DOCTOR OF MEDICINE

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Robertson, Alan

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ALLOPURINOL AS A POSSIBLE OXYGEN SPARING AGENT DURING EXERCISE IN PERIPHERAL ARTERIAL DISEASE

ALAN JAMES ROBERTSON

DEGREE OF DOCTOR OF MEDICINE

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List of abbreviations

6MWD	6 minute walk test distance
6MWT	6 minute walk test
ABI	Ankle-brachial index (formerly ankle-brachial pressure index)
ADS	Prof Allan Struthers, CI
AJR	Dr Alan Robertson, PI
ATP	Adenosine triphosphate
BMI	Body mass index
CHD	Coronary heart disease
CLI	Critical limb ischaemia
COD	Claudication onset distance (also at times referred to in the literature as ICD, initial claudication distance or CPD, claudication pain distance)
CTA	Clinical Trials Authorisation
CVD	Cerebrovascular disease
ETT	Exercise tolerance test
FMD	Flow Mediated Dilatation
GC	Gill Crowe, Vascular Nurse Specialist
GTN	Glyceryl trinitrate
HDL	High-density lipoprotein
IC	Intermittent claudication
ISRCTN	International Standard Randomised Controlled Trial
LDL	Low-density lipoprotein
LM	Mrs Lesley McFarlane, Laboratory Technician
LVEF	Left ventricular ejection fraction
LW	Dr Li Wei, Study Statistician (originally University of Dundee, now University College London)

MI	Myocardial infarction
NICE	National Institute for Health & Care Excellence
NO	Nitric oxide
PAD	Peripheral arterial disease
PVD	Peripheral vascular disease
PWD	Peak walking distance (also at times referred to in the literature as ACD, absolute claudication distance or MWD, maximum walking distance)
QALY	Quality Adjusted Life Years
QOL	Quality of life
RCT	Randomised controlled trial
ROS	Reactive oxygen species
SWT	Shuttle walk test
TASC	TAyside medical Science Centre (formerly TAHSC – Tayside Academic Health Sciences Centre)
TCTU	Tayside Clinical Trials Unit
TMF	Trial Master File
VTI	Velocity time integral
WIQ	Walking Impairment Questionnaire
XDH	Xanthine dehydrogenase
XO	Xanthine oxidase
XOR	Xanthine oxidoreductase

List of appendices

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Declaration

I hereby declare that I am the author of this thesis, that all references cited have been consulted by me and that I have carried out the work described within.

The work described in this thesis has not been previously accepted for a higher degree and I have defined the nature of my contribution to the work within the project described in the thesis.

The work contained in this thesis was carried out during my appointment as a Clinical Research Fellow in the Centre for Cardiovascular and Lung Biology, Division of Medical Sciences, Ninewells Hospital & Medical School, University of Dundee, between August 2010 and July 2012.

Signed _____ Date _____

Summary

Patients with peripheral arterial disease (PAD) can only walk so far before they get leg pain (intermittent claudication) and have to stop. They are also at risk in the future of needing amputation of one of their limbs. Allopurinol is a new possible treatment for this condition as it has been shown in coronary arterial disease to prolong exercise before angina pain occurs.

This is thought to be because allopurinol can both prevent oxygen wastage in tissues and prevent the formation of harmful oxidative stress. We hypothesised that allopurinol could prolong the time to leg pain in participants with PAD.

In a double-blind, randomised controlled clinical trial 50 participants with PAD were randomised to receive either allopurinol 300mg twice daily or placebo for six months. The primary outcome was change in exercise capacity on treadmill testing at six months. Secondary outcomes were six-minute walking distance, Walking Impairment Questionnaire, SF-36 QoL questionnaire, flow-mediated dilatation and oxidised LDL. Outcome measures were repeated mid-study and at end of study.

The mean age of participants was 68.4 years (SD 1.2) with 39/50 (78%) male. Only five participants withdrew in the course of the study, two in the active group and three in the placebo group. There was a significant reduction in uric acid levels in those on active treatment of 52.1% ($p<0.001$), but no significant change in either the pain-free or the maximum distance they were able to walk. Other measures of exercise

capacity, blood vessel function and the participants' own assessment of their health and walking ability also did not change during the course of the study.

In summary, although allopurinol has been shown to be of benefit in a number of other diseases, in this study there was no evidence of any improvement following treatment in patients with peripheral arterial disease.

1 Introduction

1.1 Peripheral Arterial Disease

1.1.1 Epidemiology

Peripheral arterial disease (PAD) is a disease that carries with it significant morbidity and through links with cardiovascular disease, mortality. The total disease prevalence has been estimated at 3-10%, rising with age to 15-20% in those over 70 years of age.[1-3] In Europe and North America alone over 27 million people are thought to be affected by it.[4] However much of this disease is asymptomatic, as demonstrated by the Edinburgh Artery Study.[5] In their study population (an age-stratified sample of men and women aged 55-74 years across ten general practices in the city) the researchers found major asymptomatic disease leading to a significant impairment in blood flow in 8% of participants, despite a prevalence of symptoms of intermittent claudication of only 4.5%. These figures for prevalence of symptomatic disease are certainly in-keeping with the 3-7% figures quoted elsewhere in the literature.[6, 7] There is an approximately two-fold increase for males compared to females, although an element of this may be due to historically higher smoking rates in men and may change slightly in the future given the relative shift in smoking habits from men to women in recent years.[7, 8]

Patients with major asymptomatic disease had a 60% increase in relative risk of cardiovascular disease.[5] The concept that asymptomatic patients with PAD were at significant mortality risk was confirmed by Criqui and colleagues when they looked at

mortality over a 10 year period in patients with PAD (the San Diego Artery Study), as illustrated by the Kaplan-Meier survival plot in Figure 1.[9]

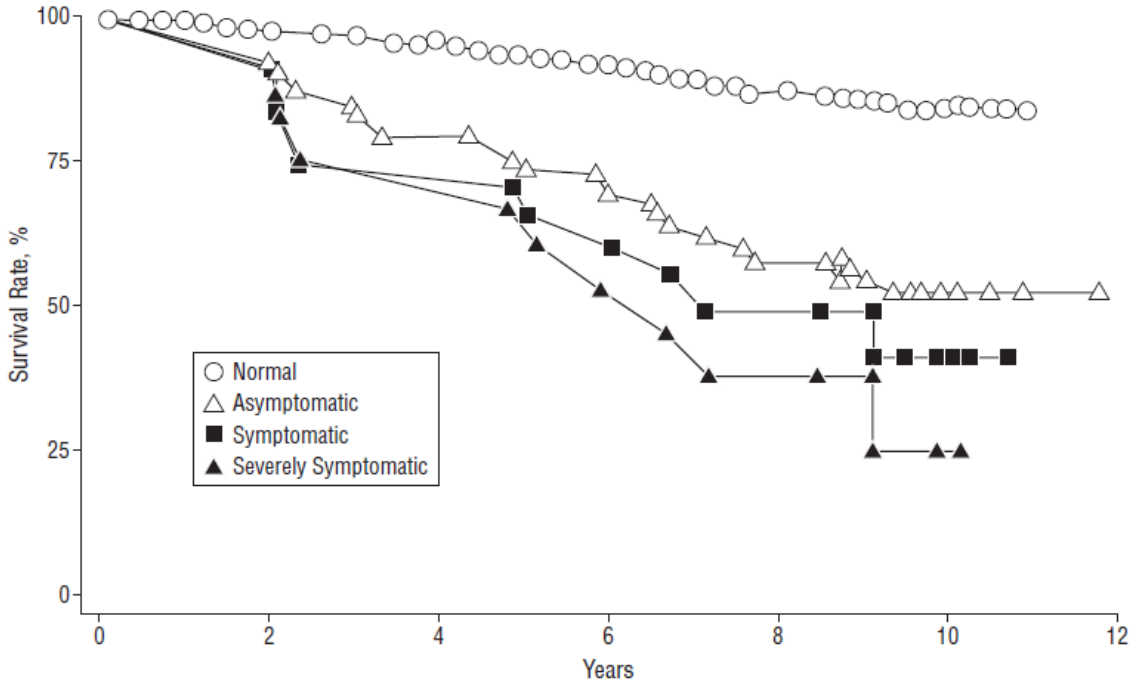

Figure 1 – Survival rate of patients with PAD by symptom, compared to non-PAD patients

The cornerstone of treatment of PAD therefore remains identification and treatment of cardiovascular risk factors, however from a patient perspective the most troubling problem remains that of intermittent claudication.[10]

1.1.2 Pathophysiology

PAD refers to the obstruction of the large arteries outwith the coronary and cerebral circulation, mainly in the lower extremities. These narrowings develop and progress through the process of atherosclerosis.[11] The clinical events resulting from atherosclerosis are directly related to the oxidation of lipids in LDLs that become trapped in the extracellular matrix of the subendothelial space. The eventual progression of the lesion is associated with the activation of genes that induce arterial

calcification, which changes the mechanical characteristics of the artery wall. These changes in the vessel wall (in particular the oxidation of lipids) predisposes to plaque rupture at sites of monocytic infiltration. Plaque rupture exposes the flowing blood to tissue factor in the lesion, and this induces thrombosis, which is the proximate cause of the clinical event.[12]

There are a number of key risk factors involved in this process of lipid infiltration, changed mechanical characteristics of the artery wall and cell damage. These include hypertension, hypercholesterolaemia, and diabetes mellitus. Analysis of data from the Framingham study provided useful information regarding the first two of these, showing that a BP of >160/95 led to a 2.5x relative increase in risk for men and 4x relative increase for women. A fasting cholesterol concentration of >7mmol/L was associated with a two-fold increase in risk of claudication.[13] It has also been shown that in patients with metabolic syndrome that the more components of the syndrome they exhibited* the worse their intermittent claudication, physical function, health-related quality of life, and peripheral circulation.[14]

With regards diabetes mellitus, the UK Prospective Diabetes Survey showed that hyperglycemia (assessed as HbA1c) was associated with an increased risk for incident PVD, independent of other risk factors including age, increased systolic blood pressure, reduced HDL cholesterol, smoking, prior cardiovascular disease, peripheral sensory

* The five key components of metabolic syndrome as defined by the National Cholesterol Education Programme Adult Treatment Panel (NCEP ATP III) consist of: (1) abdominal obesity (waist circumference >102 cm in men and >88 cm in women); (2) elevated triglycerides (≥150 mg/dL); (3) reduced high-density lipoprotein cholesterol (<40 mg/dL in men and <50 mg/dL in women); (4) elevated blood pressure (≥130/85 mm Hg); (5) elevated fasting glucose (≥110 mg/dL) as well as those with diabetes.

neuropathy, and retinopathy. Each 1% increase in HbA1c was associated with a 28% increased risk of PVD (95% CI 12–46), and each 10-mmHg increase in systolic blood pressure with a 25% increase in risk (95% CI 10–43).[15]

The relative risk of these different factors is shown in Figure 2.[1] Although careful blood glucose control for patients with diabetes and control of both hypertension and hypercholesterolaemia is certainly beneficial, the major *modifiable* risk factor for the development of PAD is smoking. This relationship has been known about for over a century now, following the observation by Erb in 1911 that intermittent claudication was three times more common amongst smokers compared to non-smokers.[1] There is a dose-response relationship with a relative risk of 3.7 in current smokers compared to 3.0 in those who had given up smoking within the past five years.[5] The link between smoking and PAD appears to be even stronger than the one between smoking and CHD.[1] Passive smokers can be adversely affected too, with repeated exposure to second-hand smoke nearly doubling the risk of development of PAD in non-smokers.[16] The failure rate for those with surgical bypass grafts is also three times higher in patients who continue to smoke, something that may be partially reversed by smoking cessation.[17]

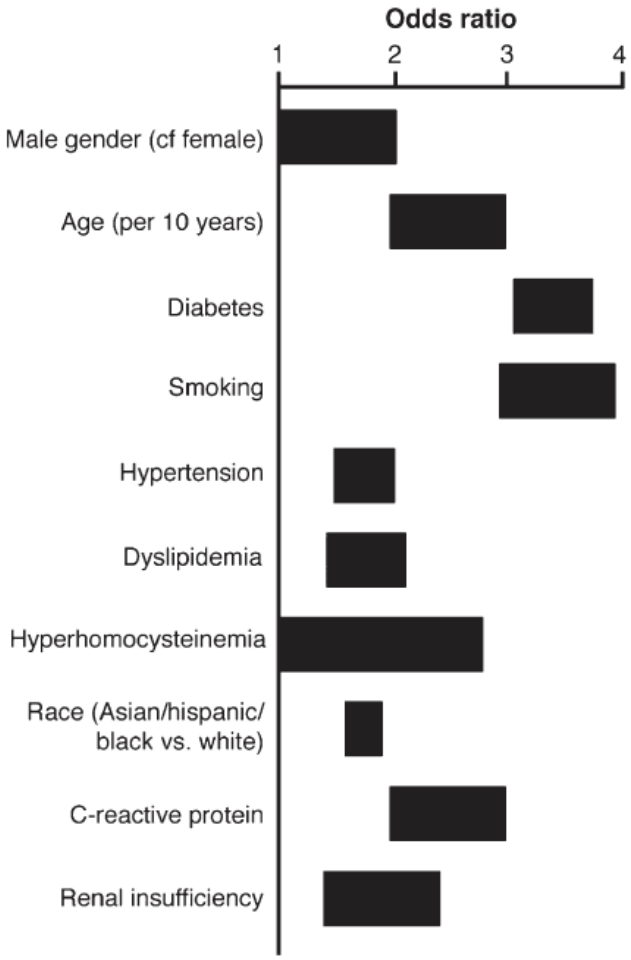

Figure 2 – Approx. range of odds ratios for risk factors for symptomatic PAD

Following a clinical suspicion of PAD, the simplest way to confirm the diagnosis is through measurement of the ankle-brachial index (ABI – also sometimes referred to as the ankle-brachial pressure index, ABPI). This involves measuring the systolic blood pressure in the brachial, posterior tibial and dorsalis pedis arteries. The highest of the leg measurements on each side is divided by the higher of the two brachial measurements, with a normal value being 1.0-1.3.[18] An ABI below 0.9 has 95% sensitivity and 100% specificity for detecting angiogram-positive PAD and is associated with a greater than 50% stenosis in at least one major blood vessel.[4] A relatively high ABI (>1.4) normally signifies calcification of the arteries and increased levels of

cardiovascular risk. Some alternate measures, such as ankle-arm index, have also been tested but none have gained the same widespread acceptance as ABI.[19]

1.1.3 Claudication pain in PAD patients

In symptomatic patients with PAD, the limited arterial supply to the lower extremities cannot meet the dynamic metabolic demand of the muscles during ambulatory activities, leading to the development of pain.[20, 21] The classical description of this in a patient with PAD is an aching muscular leg pain brought on by exertion that is rapidly relieved by rest (normally within ten minutes) – this is known as intermittent claudication. Unlike other disease processes such as osteoarthritis, there is an initial pain-free walking distance before the onset of pain.[22] Walking faster or up a steeper gradient (i.e., increasing the metabolic demand on the muscles) brings the pain on more quickly. However there is an inevitable heterogeneity between patients as to the level of exertion required to bring on pain. Interestingly the rate of symptomatic progression in PAD appears to be reasonably steady, at around 25% of patients with IC; this is most frequent during the first year after diagnosis (7%–9%) compared with 2% to 3% per year thereafter.[1] This relative stability of symptoms may be due to the development of collaterals, metabolic adaptation of ischaemic muscle, or the patient altering their gait to favour non-ischaemic muscle groups.[1] It may also be due to patients altering their physical activity levels to compensate – for example by walking more slowly.[23, 24]

The Fontaine scheme classifies four stages of peripheral arterial disease. Peripheral arterial disease can be asymptomatic (Fontaine stage I) or symptomatic (Fontaine

stages II–IV). The most common symptom of peripheral arterial disease is intermittent claudication (Fontaine stage II), which is characterised by pain in the legs or buttocks that occurs with exercise and is relieved with rest. Two further stages exist: pain in the extremities at rest (ischaemic rest pain, Fontaine stage III) and necrosis and gangrene (Fontaine stage IV).[25]

Patients with IC often experience a diminishing quality of life as their walking speed and range worsen, leading to progressively limited mobility and independence and the consequent detrimental effect on their mental health and well-being.[4, 26, 27] However the claudication symptoms of PAD are not as well-recognised by the general public (or indeed at times the medical profession) compared to angina, the analogous symptom for CHD.[28] This tends to lead to a delay in seeking medical help (population screening studies have suggested between 10-50% of patients with symptoms of IC have not sought medical help regarding their symptoms[1]) with the consequent progression of disease (and risk of other cardiovascular events) in the meantime.

The location of the claudication symptoms tends to correspond with the source of the arterial lesion, as outlined in Table 1[18] and Figure 3[22].

Artery	Symptoms
Aortoiliac (Leriche syndrome)	Buttock, hip and in some cases thigh claudication; bilateral severe aortoiliac disease can cause impotence in men
Common femoral	Thigh, calf claudication or both; patients have normal groin pulses but diminished pulses distally
Superficial femoral	Upper two-third of calf claudication
Popliteal	Lower third of calf claudication
Tibial or peroneal	Isolated foot claudication is rarely seen with atherosclerotic occlusive disease but is commonly seen with *thromboangiitis obliterans* (Buerger's disease)

Table 1 – Correlation between claudication symptoms and lesion location

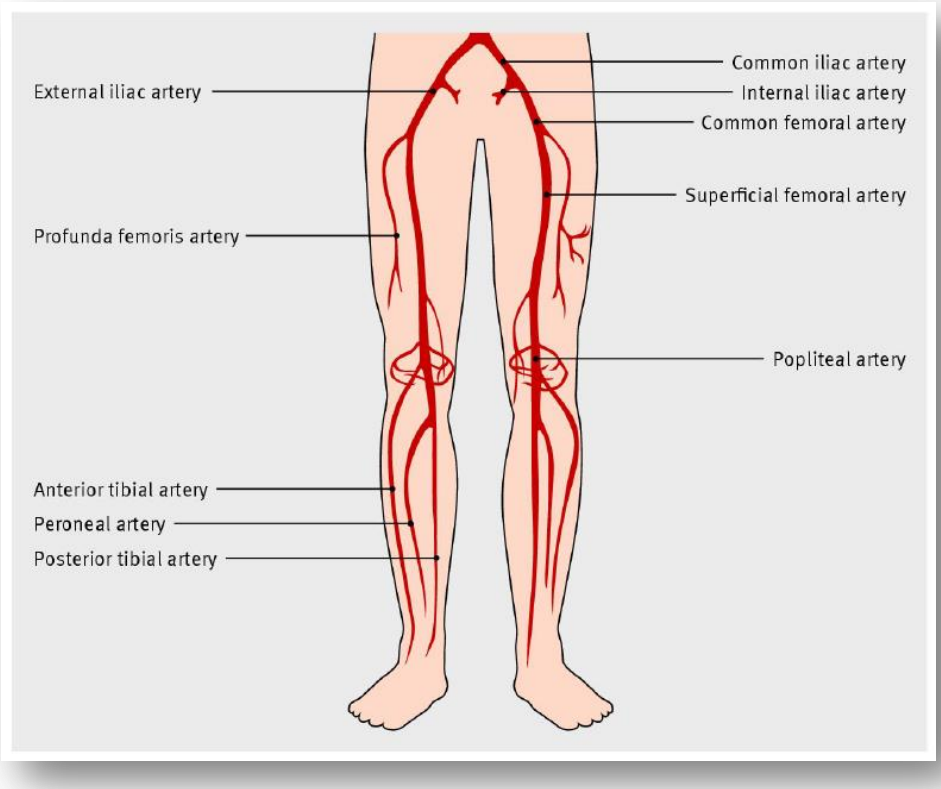

Figure 3 – Arteries of the lower limb

This pain is due to build-up of anaerobic metabolites, mainly lactic acid.[29] This is formed through the ischaemic cascade.[30] The lack of oxygen delivery to the myocytes leads to failure of the normal processes for ATP formation and for cells to switch to anaerobic metabolism, producing lactic acid. The ATP-reliant cell membrane transport pumps also fail, leading to rising intracellular Ca^{2+} levels. This excess calcium leads to the generation of free radicals, reactive oxygen species and calcium-dependent enzymes such as calpain, endonucleases, ATPases, and phospholipases in a process called excitotoxicity.[31-33] The reduction in ATP production also leads to inefficient use of the electron transport chain in the mitochondria, with a consequent reduction in superoxide dismutase and increase in reactive oxygen species.[34]

1.1.4 Disease progression

The most worrying complication of PAD is critical limb ischaemia (CLI). This is characterised by ulceration, gangrene, or rest pain consistently for more than two weeks. This should be differentiated from benign night-time calf cramps – true rest pain usually affects the toes or foot of the affected limb and patients often describe hanging the affected limb out of bed to ease symptoms.[22] Thankfully this is a relatively rare outcome of claudication, with only 1% to 3.3% of patients with IC needing major amputation over a five-year period.[1] Those with diabetes and those who continue to smoke are however at significantly increased risk. Figure 4 shows the approximate magnitude of the effect of different risk factors on the development of CLI.[1]

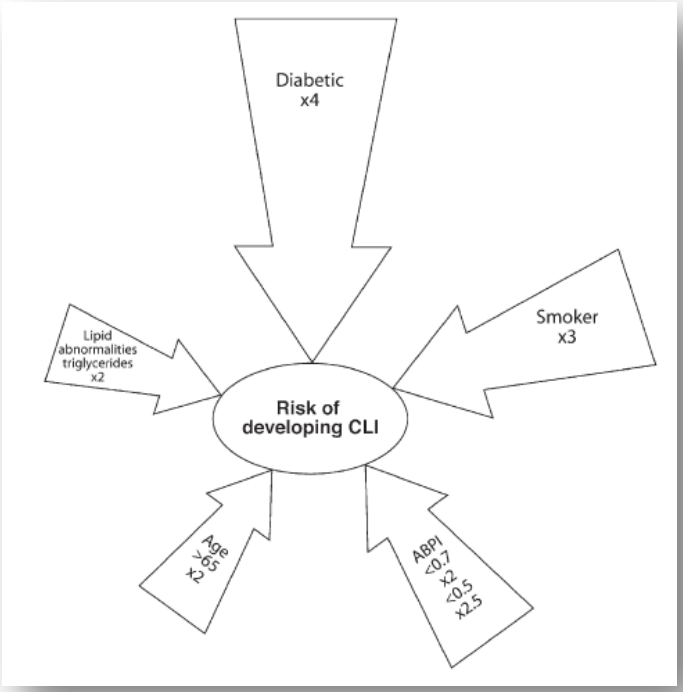

Figure 4 – Approx. magnitude of the effect of risk factors on development of CLI

Interestingly, although some patients will experience a gradual progression of symptoms (from asymptomatic PAD through to CLI), more than half of patients undergoing amputation for CLI were not troubled by ischaemia in the six months prior to surgery.[35] However patients with PAD often have significant comorbidities, therefore the lack of ischaemic symptoms in these patients may also be a reflection of their limited physical mobility.[22]

Unsurprisingly there is also a great overlap between PAD and atherosclerosis of other parts of the circulatory system, particularly the coronary and cerebrovascular circulation. The REACH Registry (67,888 patients) provides some excellent information regarding this overlap between circulatory system disease, as illustrated in Figure 5.[36, 37]

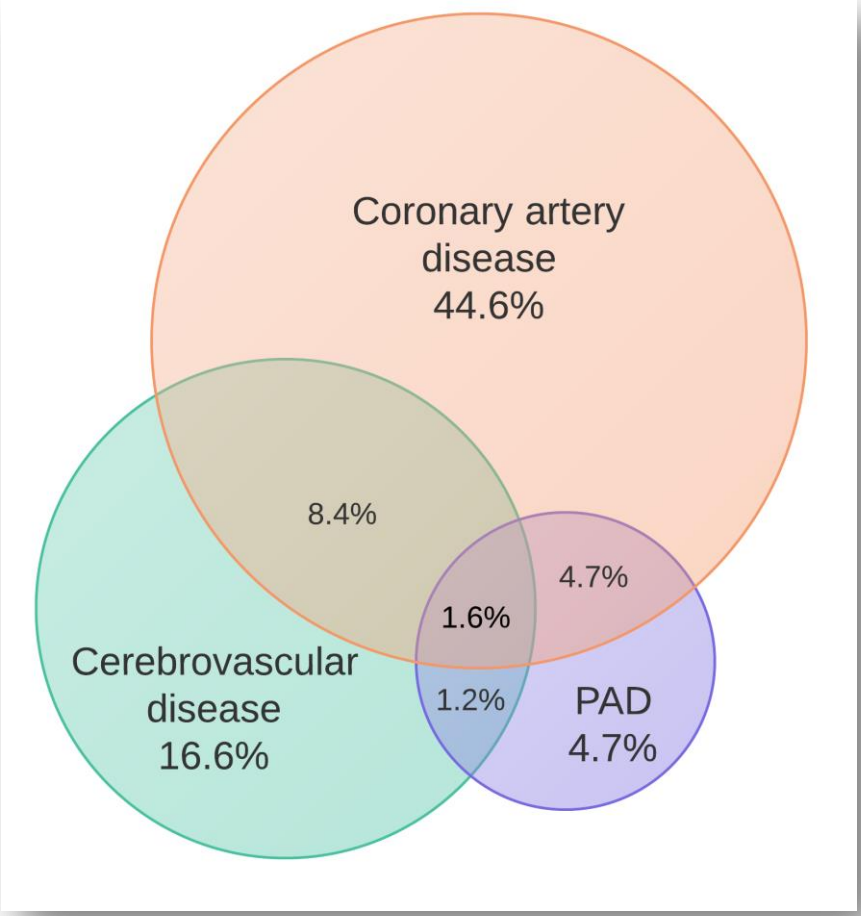

Figure 5 – Overlap of vascular disease in different arterial systems (created from REACH study data)

As a result of this overlap it is estimated that 2-4% of PAD patients have a non-fatal cardiovascular event every year. The risk is higher in the first year after developing IC, but remains higher at all times than the risk of ever requiring amputation for leg ischaemia.[38]

The mortality of patients with known PAD (mainly those with IC) is quoted at 30% at five years, 50% at ten years, and 70% at 15 years, without any clear improvement in

these figures in the past few decades. The overall mortality is approximately two-and-a-half times that of an age-matched general population.[38]

1.1.5 Therapeutic options

As highlighted above, the elevated mortality risk in patients with PAD is mainly due to increased deaths from cerebrovascular disease (CVD) and coronary heart disease (CHD). Criqui *et al* demonstrated a RR of 3.1 of all-cause mortality in PAD patients, rising to 5.9 when looking at death due to CVD and 6.6 for death due to CHD.[9] This increase in risk persisted even in patients without known pre-existing CHD, albeit falling slightly to 4.3.

Cardiovascular risk factor modification therefore remains a mainstay of treatment, with an antiplatelet agent such as aspirin or clopidogrel being recommended.[1, 10, 39] Although the benefits of anti-platelet therapy may not be as marked for patients without co-existing CVD, an overall odds reduction of around one-fifth has been shown across all subgroups of patients with PAD[40], making it a good baseline treatment. The CAPRIE study (an RCT of 19,185 patients with all forms of cardiovascular disease) found clopidogrel to be more effective than aspirin, with a relative risk reduction of 8.7% for MI, stroke and vascular death.[22, 41] A subgroup analysis of patients with PAD indicated a 23.8% relative risk reduction in favour of clopidogrel. There had previously been cost concerns regarding prescription of clopidogrel relative to aspirin, however in June 2009 it came off-patent in the EU and equally efficacious generic forms were licensed.[42, 43]

Other risk factors such as hypertension, hypercholesterolaemia and screening/treatment for diabetes all also need addressed.[44] As well as reducing cardiovascular risk these treatments can sometimes have a positive effect on claudication symptoms. In particular for statin therapy there is evidence that high dose simvastatin and atorvastatin can lead to improved 6-minute walking distance and walking velocity.[45-47] The risk factor modification that can bring the greatest benefit to patients though is smoking cessation – it is critical that this is clearly explained to any patient diagnosed with PAD.

However in symptomatic patients, once risk factor modification has taken place there remains the problem of the claudication pain. To address this there are three main therapeutic options – exercise programmes, pharmacological therapy and intervention (through either the endovascular or surgical approach).

Hiatt *et al* produced a flowchart for therapies in PAD that has also received the backing of the Trans-Atlantic Inter-Society Consensus document taskforce (TASC II) and is shown in Figure 6.[1, 48]

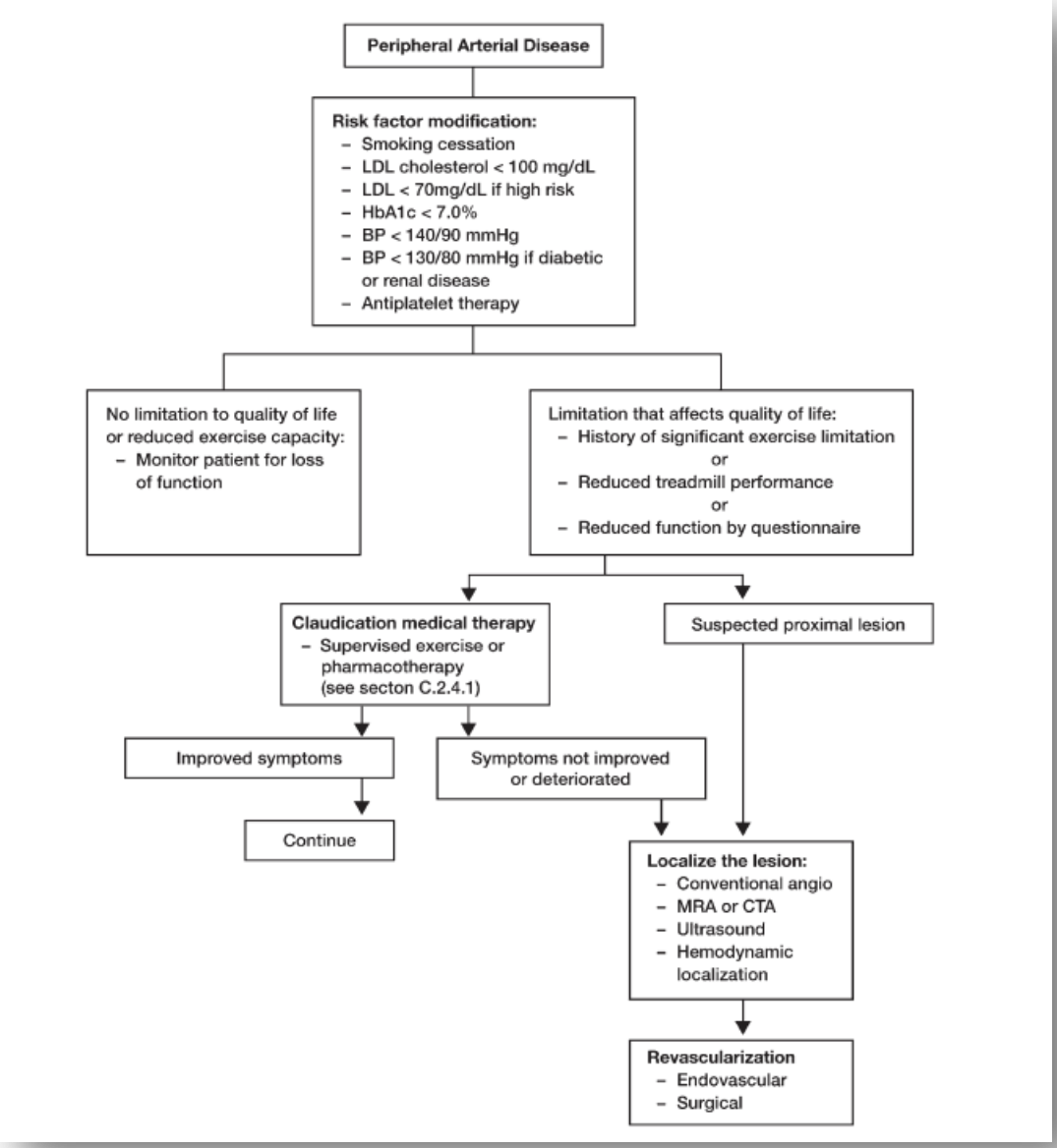

Figure 6 – Flowchart of therapy selection in PAD

Exercise

This treatment category has historically been quite heterogeneous, ranging from providing standardised advice to patients to "take more exercise", to one-on-one counselling sessions, to regular supervised exercise classes using treadmills.[49] There is evidence that supervision helps in the achievement of optimal results and this can be

to quite reasonable clinical benefit.[50] One large trial showed an approximately 150m increase in walking distance (from a baseline of 300m), with an effect size of 0.58 at three months, maintained to 0.89 at six months.[1, 51] There is inadequate evidence at present to attribute the functional benefit from exercise to the growth of new arterial collaterals (angiogenesis); in contrast, clinical improvement is more likely to be due to alterations in skeletal muscle metabolism, muscle hypertrophy, improvements in endothelial function, improved cellular or subcellular function, or altered gait.[52-54]

A Cochrane Review in 2008 found 22 randomised controlled trials (involving a total of 1,200 participants) of exercise regimens in patients with IC due to peripheral arterial disease.[55] There was some variation in the exercise regimens used, however all recommended at least two sessions weekly of mostly supervised exercise. Quality of the included trials was good, though the majority of trials were small with 20 to 49 participants. Compared with usual care or placebo, exercise significantly improved maximal walking time: mean difference (MD) 5.12 minutes (95% confidence interval (CI) 4.51 to 5.72;) with an overall improvement in walking ability of approximately 50% to 200%; exercise did not affect the ankle brachial index (ABI) (MD -0.01, 95% CI -0.05 to 0.04). Walking distances were also significantly improved: pain-free walking distance MD 82.19 metres (95% CI 71.73 to 92.65) and maximum walking distance MD 113.20 metres (95% CI 94.96 to 131.43). Improvements were seen for up to two years.

However the Cochrane team did comment that the evidence was generally limited for exercise compared with more interventional treatments (most of the studies just compared exercise to best medical care). A meta-analysis in 2011 compared

endovascular and non-invasive therapies in treatment of intermittent claudication and found that supervised exercise can provide similar results to endovascular therapy but that combining both approaches can have a synergistic effect.[56] Greenhalgh *et al* showed a small but statistically significant improvement in ABI for those receiving supervised exercise alone vs supervised exercise plus endovascular therapy (0.74 vs 0.90, $p<0.05$).[57] However the study authors themselves questioned the representativeness of their patient population given the low recruitment rate and low rate of eligibility. The issue should hopefully be more comprehensively addressed in a prospective, multicenter, randomized, controlled clinical trial to investigate the relative merits of exercise and endoluminal revascularisation that is currently underway. The CLEVER (Claudication: Exercise Vs. Endoluminal Revascularization) study, is funded by the Heart, Lung, and Blood Institute of the National Institutes of Health and aims to evaluate the relative efficacy, safety, and health economic impact of four treatment strategies for people with aortoiliac peripheral arterial disease and claudication. The treatment arms are: (1) optimal medical care (claudication pharmacotherapy); (2) primary stent placement; (3) supervised exercise rehabilitation; and (4) combined stenting with supervised exercise rehabilitation. It is a five-year study across 25 centres in the United States and is aiming to monitor approximately 250 patients for their responses to treatment during an 18-month follow-up period.[58, 59]

Recruitment to the CLEVER study is now complete and final results are awaited.[60] Initial results from six months of follow up showed that supervised exercise resulted in superior treadmill walking performance compared to revascularisation with stenting, even for those with aortoiliac peripheral artery disease. Interestingly however,

although disease-specific quality of life as assessed by WIQ and Peripheral Artery Questionnaire both improved compared to optimal medical therapy with either exercise or stenting, the extent of the improvement was greater in patients who had undergone stenting.[61] Unfortunately their initial report does not cover analysis of the cohort that received both supervised exercise plus stenting.

Intervention

The main role for endovascular and surgical intervention is for patients with suspected proximal artery lesions (findings of buttock claudication, reduced/absent femoral pulse), ongoing symptoms limiting their quality of life despite non-invasive therapy, or for those with critical limb ischaemia.[1, 53] In patients with milder disease a meta-analysis of endovascular (angioplasty with/without stenting) and non-invasive therapies found no evidence that endovascular therapy alone provides improved outcome over supervised exercise alone.[56] However it should be noted that the medical treatments in the studies that were considered were limited to advice on managing risk factors, aspirin (or clopidogrel in many cases) and occasionally smoking cessation advice. There was no concerted attempt at either an exercise programme or consideration of other pharmacological therapy. As mentioned above, the CLEVER study will hopefully provide a more comprehensive comparison between exercise and intervention and help guide clinicians as to the best time to pursue an interventional strategy.

Restenosis by myointimal hyperplasia after peripheral arterial angioplasty or stenting is a major problem limiting its long-term efficiency and patency, and may lead to

recurrent symptoms. There is some evidence that drug-eluting stents and balloons may be of benefit, but they are more expensive and cost-effectiveness has not yet been proven.[62]

Over time the improvement in percutaneous experience and technology has led to a reduction in the need for open surgery. It remains an option, but in many cases a percutaneous approach is feasible. This has been summarised by Weinberg *et al*. in the flowchart shown in Figure 7.[63]

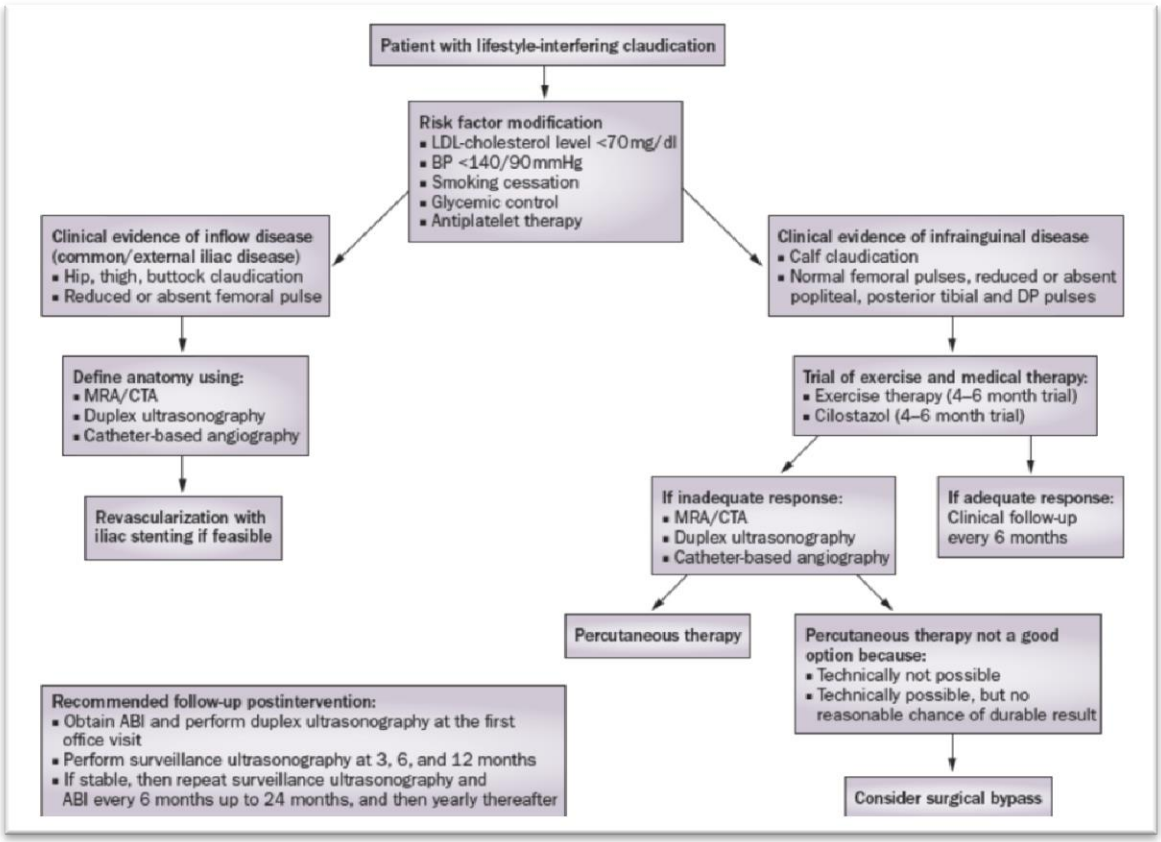

Figure 7 – Approach for the patient who presents with claudication that interferes with their lifestyle

Pharmacological

The three main pharmacological options for treatment of claudication symptoms that have received reasonably wide acceptance currently consist of cilostazol, pentoxifylline and naftidrofuryl.[64] The comparative efficacy of these treatments compared to exercise (and atorvastatin) is shown in Figure 8.[53]

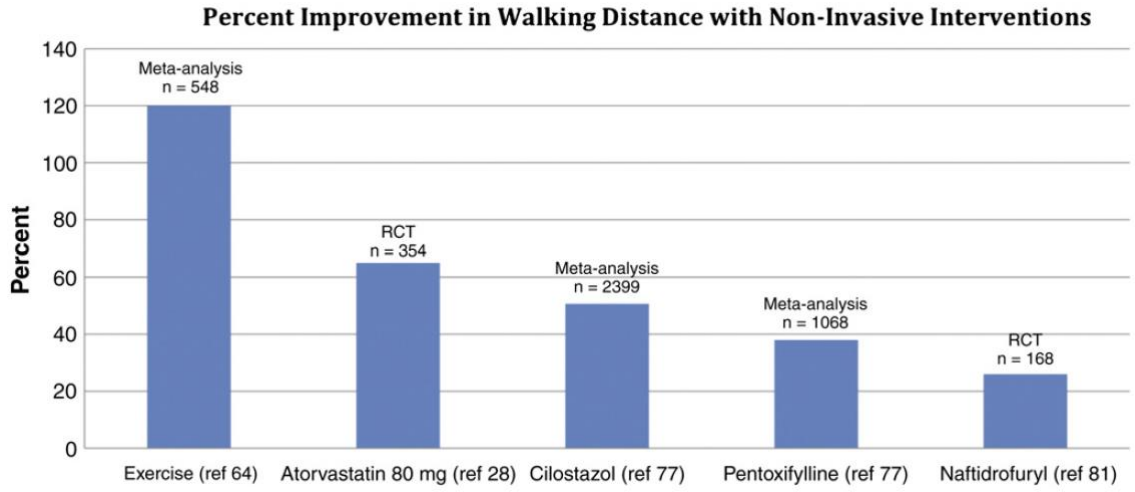

Figure 8 – Improvement in walking distance with non-invasive interventions

Cilostazol is a type III phosphodiesterase inhibitor with a mix of vasodilator, metabolic and antiplatelet activity.[65, 66] It has shown consistent evidence of moderate benefit[67] – usually in the region of 50-70m improvement on peak walking distance on treadmill.[68] The study by Regensteiner *et al* also looked at quality of life and found a significant overall improvement in both SF-36 and WIQ scores. Patients receiving cilostazol did however consistently report an increased number of side-effects compared to those on placebo.[69] These included diarrhoea, headache and palpitations.[70] As it is in the same class as milrinone there is concern regarding a possible increase in mortality in patients with known congestive cardiac failure and its use is contra-indicated in this

group. However in other patient groups to date there has been little evidence of any direct increase in mortality rates from cilostazol use. Postmarketing surveillance in the United States, representing 70,430 patient-years of cilostazol exposure, showed minimal accounts of myocardial infarction, stroke, or death.[71]

Pentoxifylline is a methylxanthine derivative that is known to increase erythrocyte deformability, inhibit neutrophil adhesion and activation and potentially lower plasma fibrinogen concentrations. It was hoped these changes would improve claudication symptoms but the trial results have been extremely variable. A recent Cochrane review of 23 studies (covering 2,816 participants) found great heterogeneity between the interventions and results.[72] Only 17 of the studies compared pentoxifylline with placebo and in those the outcomes varied greatly, with the difference in percentage improvement in total walking distance ranging from 1.2% to 155.9%, and for pain-free walking distance the difference ranging from -33.8% to 73.9%. They were unable to comment on the statistical significance of these changes due to lack of data, but found significant difference in ankle brachial pressure index after treatment with pentoxifylline. The one positive for pentoxifylline is that it is generally well tolerated and therefore the current ACC/AHA PAD guidelines only recommend its use as an alternative in patients who are unable to tolerate cilostazol due to side-effects or in whom the use of the latter is contraindicated.[52, 73]

Naftidrofuryl is a 5-hydroxytryptamine type 2 agonist and has been in widespread use for over 20 years in treating intermittent claudication, more so in Europe than North America.[53] As well as reducing erythrocyte and platelet aggregation it is thought it

may have positive effects on muscle metabolism.[74] A large number of trials have been carried out and in general it appears to be well tolerated and leads to a modest improvement in walking distance by around 26%.[75-77] There is also some evidence on improved quality of life with naftidrofuryl, particularly in the domains of daily living, pain and social life.[78, 79] Intravenous delivery has been evaluated but found only to show a trend to symptom improvement, with no statistically significant change.[80]

However although these agents may have demonstrated clinical effectiveness to varying degrees, there are major concerns regarding cost-effectiveness. A recent NICE technology appraisal recommended only naftidrofuryl as an option for treatment of intermittent claudication, with cilostazol and pentoxifylline falling into the "not recommended" category. One of the main reasons for their decision was that these three drugs have no survival benefit – thus the Quality-Adjusted Life Year (QALY) calculations they carried out were mainly driven by the gain in mobility. Given the small improvement in pain-free walking distance (around 5-30%, varying by study) that they provide and comparing to placebo, NICE calculated values of £50,700 (cilostazol), £6,070 (generic naftidrofuryl), £11,060 (branded naftidrofuryl) and £54,800 (pentoxifylline) per QALY.[25]

One further, naturally occurring, remedy that has been at times considered for PAD is garlic. Medicinal use of garlic goes back to Egyptian times and there has been some evidence for the use of commercially available preparations in reducing atherosclerotic risk factors, especially cholesterol and platelet aggregation. However a systematic review by the Cochrane Collaboration found only one suitable trial – this showed no

statistically significant change in walking distance.[81] It is interesting that this topic was chosen by the Collaboration as an area that deserved a review to be carried out – this perhaps underscores the difficulties in finding effective treatments for PAD.

Novel therapies

In addition to the core approaches of exercise, intervention and pharmacology discussed above, there have been some interesting developments by way of biological therapies. These mainly consist of utilising various angiogenic growth factors or autologous stem cells in an attempt to improve perfusion in areas of ischemia through the development of new blood vessels from pre-existing blood vessels. This process has been termed therapeutic angiogenesis and involves capillaries in the size range of 100 to 300μm.[82, 83] Due to the short half-life of most recombinant proteins the majority of clinical trials have focused on either stem cell or gene therapy approaches to provide longer-term delivery of these angiogenic growth factors.

One group of patients in which there is particular interest in biological therapies is in those with critical limb ischaemia, especially as almost half of such patients are not candidates for open revascularization – either due to unsuitable anatomy, or associated comorbidities.[84] Although some will be suitable for percutaneous intervention there remains a significant number in which no clear treatment options are available, thus ultimately leading to major amputation to control pain or infection. Medical therapy for CLI is limited to anti-platelets and secondary prevention (along with good wound care), making biological therapies all the more desirable. In addition, the majority of patients with CLI have developed multilevel, complex vascular disease

with no one easy target to repair. Biological therapies offer the potential to target all affected vessels simultaneously, potentially improving the overall treatment response.

Stem cell therapy involves obtaining mononuclear cells from either the patient's bone marrow or peripheral blood following subcutaneous injections of granulocyte colony-stimulating factor for a number of days. These cells are then treated in a laboratory and injected (often at multiple sites) into the ischaemic lower limb(s). The advantage of this technique is its relative simplicity and familiarity as a process from the field of Haematology. There are also fewer concerns regarding 'off-target' angiogenesis compared to gene therapy. Although there have been a number of positive small trial reports showing improvements in pain-free walking distance and reductions in amputation, a Cochrane review in 2011 felt that evidence from larger randomised controlled trials was needed in order to provide adequate statistical power to assess the role of intramuscular mononuclear cell implantation in patients with CLI.

Gene therapy involves the insertion of genetic material into dysfunctional cells or tissues with the goal of curing the disease or alleviating its burden.[85] Trials of gene therapy using a number of potential target factors have been carried out to date, with the main ones being: vascular endothelial growth factor (VEGF), hypoxia-inducible factor 1α, and fibroblast growth factor (FGF). A summary of the major trials to date is shown in Figure 9 below.[85]

Reference	Year	Disease	Gene	Delivery	Vector	No. of patients	Total dose	No. of doses
Phase I								
Isner *et al.*[15]	1996	CLI	VEGF	IA	Plasmid	1	2 mg	1
Baumgartner *et al.*[16]	1998	CLI	VEGF	IM	Plasmid	9	4 mg	2
Isner *et al.*[17]	1998	CLI	VEGF	IM	Plasmid	6	2–4 mg	2
Simovic *et al.*[18]	2001	CLI	VEGF	IM	Plasmid	29	3–9 mg	2–3
Shyu *et al.*[19]	2003	CLI	VEGF	IM	Plasmid	21	0·8–5·2 mg	2–3
Kim *et al.*[20]	2004	IC/CLI	VEGF	IM	Plasmid	9	2–8 mg	2
Rajagopalan *et al.*[21]	2001	IC/CLI	VEGF	IM	Virus	5	4×10^8 to 4×10^{10} units	1
Rajagopalan *et al.*[22]	2002	IC	VEGF	IM	Virus	18	4×10^8 to 4×10^{10} units	1
Comerota *et al.*[23]	2002	CLI	FGF	IM	Plasmid	51	0·5–16 mg	1
Rajagopalan *et al.*[24]	2007	CLI	HIF-1α	IM	Virus	34	1×10^8 to 2×10^{11} units	1
Morishita *et al.*[25]	2004	CLI	HGF	IM	Plasmid	6	4 mg	2
Phase II								
Mäkinen *et al.*[26]	2002	IC/CLI	VEGF	IA	Virus/plasmid	54	2 mg; 2×10^{10} units	1
Rajagopalan *et al.*[27]	2003	IC	VEGF	IM	Virus	105	4×10^9 to 4×10^{10} units	1
Kusumanto *et al.*[28]	2006	CLI	VEGF	IM	Plasmid	54	4 mg	2
Nikol *et al.*[29]	2008	CLI	FGF	IM	Plasmid	125	16 mg	4
Powell *et al.*[30]	2008	CLI	HGF	IM	Plasmid	104	1·2–12 mg	2–3
Grossman *et al.*[31]	2007	IC	Del-1	IM	Plasmid	105	42 mg	1
Phase III								
Belch *et al.*[32]	2011	CLI	FGF	IM	Plasmid	525	16 mg	4

Figure 9 – Gene therapy trials 1996-2011 for PAD

Unfortunately to date, despite promising Phase II results, the overall clinical results for gene therapy have not been positive. The large TAMARIS trial of FGF (Phase III, 525 patients) in patients with CLI found no difference between active and placebo treatment groups, with major amputation or death in 86 patients (33%) in the placebo group, and 96 (36%) in the active group (hazard ratio 1.11, 95% CI 0.83-1.49; p=0.48).[86] It was noted that this was in striking comparison to the positive results of the Phase II TALISMAN trial.[87] When the results of these two trials were compared, the combined 1-year major amputation and death rates in both treated groups were similar (27 per cent TALISMAN and 36 per cent TAMARIS). Those reported in the control groups, however, differed markedly (52 per cent TALISMAN and 33 per cent TAMARIS). It has therefore been postulated that the deemed efficacy of NV1FGF in TALISMAN was less due to therapeutic angiogenesis, and more the consequence of an abnormally high placebo endpoint rate not representative of the CLI population. Additionally, TAMARIS reported the use of current guidelines to ensure optimal medical management of

included patients, whereas there was no evidence of best medical treatment being used in TALISMAN, which may have further contributed to the discrepancy between the trials.[85, 88]

1.2 Oxidative stress

An inevitable consequence of normal intracellular metabolism is the production of reactive oxygen species (ROS). This term encompasses a wide range of molecules, including superoxide anions ($O_2^{\bullet -}$), hydroxyl radicals ($^{\bullet}OH$) and hydrogen peroxide (H_2O_2). The key element these molecules have in common is the presence of an unpaired electron (often leading to the terminology 'free radical') and thus extremely high chemical reactivity.[89] Oxygen as a molecule is especially susceptible to radical formation due to the presence of two unpaired electrons in separate orbitals in its outer shell.[90]

In addition to endogenous sources from metabolism there are also a number of external stimuli that may lead to the generation of these molecules – for example exposure to ionising radiation, toxins (such as cigarette smoke), chemotherapeutic agents and ultraviolet light. Cells exposed to abnormal environments such as hyperoxia or hypoxia also have much higher rates of ROS generation.

Given the highly reactive nature of these molecules they have the ability to cause both direct cell damage and to interact with cell signalling pathways. In combination this has the potential to lead to ageing, age-related diseases (including cancer) and cell death.[91]

One of the best-known toxic effects of ROS is lipid peroxidation, involving the incorporation of a radical species (e.g., a hydroxyl radical) into the unsaturated fatty acid, as depicted in Figure 10. This membrane lipid peroxidation may have a number

of deleterious effects, such as increased membrane rigidity, decreased activity of membrane-bound enzymes (such as sodium pumps), altered activity of membrane receptors and altered permeability.[90]

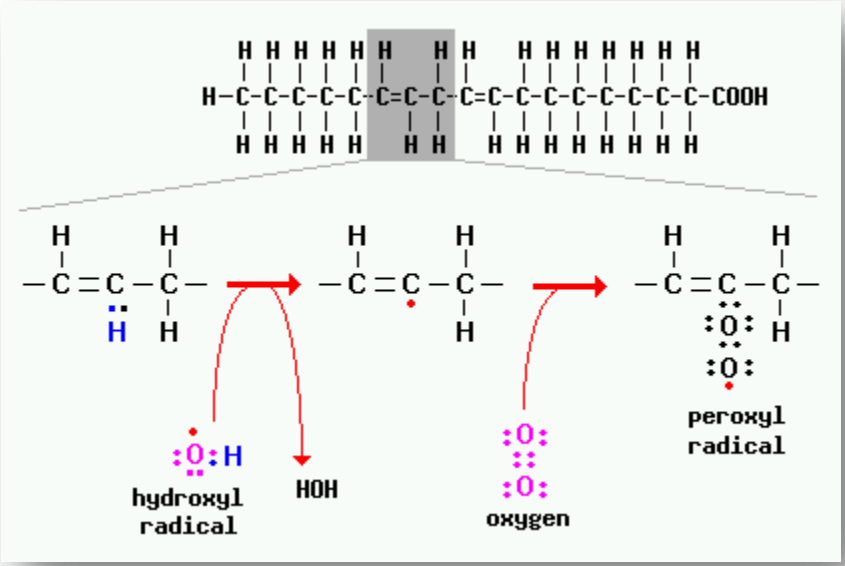

Figure 10 – Illustration of lipid peroxidation (courtesy R Bowen)

Mammalian physiology has therefore evolved both enzymatic and non-enzymatic mechanisms to manage and defend against these potentially damaging molecules, as outlined in Table 2.[90, 92, 93] It has been noted that exercise appears to increase ROS, however training seems to reduce the oxidative stress of exercise, with trained athletes showing less evidence of lipid peroxidation and an enhanced defence system compared to untrained subjects.[94]

Enzymatic defences	
superoxide dismutases (SOD)	• enzymes that catalyse the conversion of two superoxide molecules into hydrogen peroxide and oxygen ($O_2^{\bullet-} + O_2^{\bullet-} \rightarrow H_2O_2$) • although hydrogen peroxide is still a ROS it is substantially less toxic that superoxide • SOD accelerate this detoxifying reaction roughly 10,000-fold over the non-catalysed reaction • these enzymes depend on a bound manganese, copper or zinc for their antioxidant activity. In mammals, the manganese-containing enzyme is most abundant in mitochondria, while the zinc or copper forms predominant in cytoplasm • SOD are inducible enzymes - exposure of bacteria or vertebrate cells to higher concentrations of oxygen results in rapid increases in the concentration of SOD
Catalase	• found in peroxisomes in eukaryotic cells • degrades hydrogen peroxide to water and oxygen, hence completing the detoxification reaction started by SOD
glutathione peroxidase	• group of enzymes, the most abundant of which contain selenium • like catalase, these enzymes degrade hydrogen peroxide • also reduce organic peroxides to alcohols, providing another route for eliminating toxic oxidants
Other enzymes	• glutathione transferase, caeruloplasmin, hemoxygenase
Non-enzymatic defences	
Vitamin A	• obtained from the diet: green and yellow vegetables, dairy products, fruits and organ meats • within the body, vitamin A can be found as retinol, retinal and retinoic acid (all toxic at high concentrations and thus stored as long chain fatty esters/provitamin carotenoids in the liver, kidney and adipose tissue • antioxidant activity of vitamin A and

	carotenoids is conferred by the hydrophobic chain of polyene units that can quench singlet oxygen , neutralize thiyl radicals and combine with and stabilize peroxyl radicals [93] • some evidence that vitamin A deprivation leads to increased oxidative stress [92]
Vitamin C	• a water-soluble antioxidant that can reduce radicals from a variety of sources • also appears to participate in recycling vitamin E radicals • under certain circumstances can also act as a pro-oxidant
Vitamin E	• the major lipid-soluble antioxidant • plays a vital role in protecting membranes from oxidative damage • primary activity is to trap peroxyl radicals in cellular membranes • some evidence of benefit of dietary supplementation in helping to reduce exercise-induced muscle damage[95]
Glutathione	• possibly the most important intracellular defence against damage by reactive oxygen species • tripeptide composition (glutamyl-cysteinyl-glycine) - the cysteine provides an exposed free sulphydryl group (SH) that is very reactive, providing an abundant target for radical attack • reaction with radicals oxidizes glutathione, but the reduced form is regenerated in a redox cycle involving glutathione reductase and the electron acceptor NADPH • some evidence that intravenous infusion may improved microcirculation and symptoms in PAD[96]
Other (small molecules that function as anti-oxidants)	• bilirubin • uric acid (see section 1.3) • flavonoids and carotenoids

Table 2 – Enzymatic and non-enzymatic defences against ROS

It should be noted however, as is often the case in physiology, that a balance must be struck when dealing with the regulation of ROS. Too low a level of these molecules may lead to impairment in both cell proliferation and host defences – for example, generation of ROS by phagocytic cells is a key defence mechanism to combat infective organisms and release of ROS by platelets involved in wound repair acts as a chemo-attractant for other platelets.

This effect on platelets has been looked at in more detail by Davi *et al*.[97] They examined lipid peroxidation and platelet activation in obese women and investigated whether this was modifiable after body weight reduction. The study consisted of women who were in good general health with a normal medical history/physical examination save for a group of 44 participants who had android obesity (BMI >28; waist-to-hip ratio of ≥0.86) and 25 who had gynoid obesity (BMI >28; waist-to-hip ratio of ≤0.86). A non-obese comparator group of 24 women all had a BMI lower than 25. To minimise confounding they excluded any women with a history or evidence of atherothrombotic diseases, diabetes mellitus, cigarette smoking, dyslipidaemia, current/recent pregnancy, arterial hypertension or were receiving hormone replacement therapy or aspirin.

They found that android (abdominal) obesity was associated with a four-fold higher rate of thromboxane metabolite excretion (a marker of platelet activation) than measured in non-obese women, of a comparable magnitude to the changes found in association with type II diabetes mellitus, cigarette smoking and hypercholesterolaemia. They postulated a biochemical link for this through increased

levels of F_2-isoprostanes. These bio-eicosanoids are produced from arachidonic acid through a process of non-enzymatic free radical-catalysed lipid peroxidation, thus giving a marker of the effects of oxidative stress (Figure 11).[97]

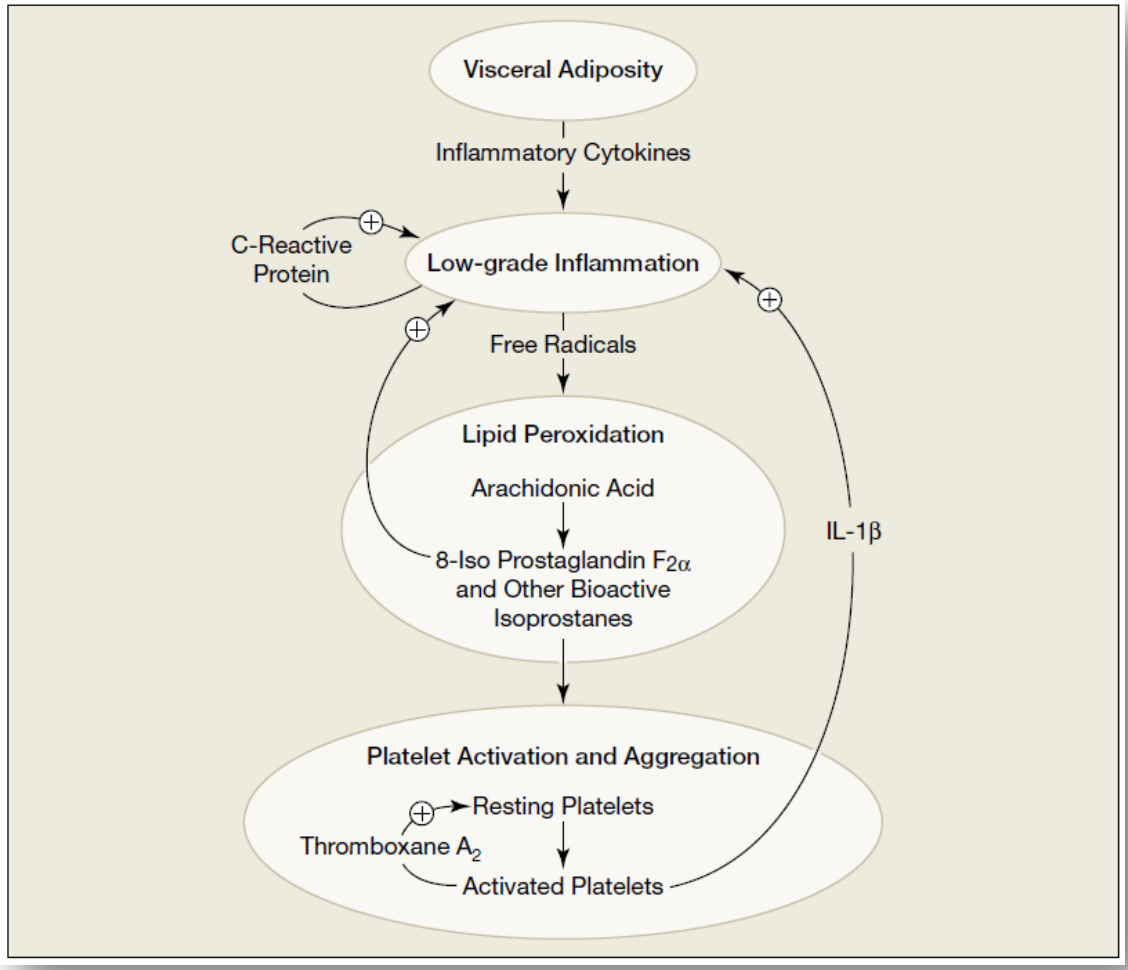

Figure 11 – platelet activation through inflammation and nonenzymatic peroxidation of arachidonic acid

The research team then proceeded to obtain evidence for a cause-and-effect relationship via a short-term diet-induced weight loss programme. They found that a 10% reduction in body weight obtained through a 12-week programme was associated with a reduction in thromboxane biosynthesis of over 50% and normalisation of this

non-invasive index of platelet activation. It was also associated with statistically significant reductions in urinary 8-iso $PGF_{2\alpha}$ isomer, a reflection of the in-vivo formation of F_2-isoprostanes. The study was however self-selecting with no randomisation to weight loss vs maintenance (albeit there were some who failed to lose weight and had no reduction in their biomarkers) and had a small sample size. It does however raise interesting questions around the role of central obesity in platelet activation and potentially an insight into the benefits of reducing lipid peroxidation through other routes in addition to weight loss.

An overview of both the sources of ROS and the homeostatic balance required to maintain appropriate levels is shown in the illustration by Finkel & Holbrook in Figure 12.[91]

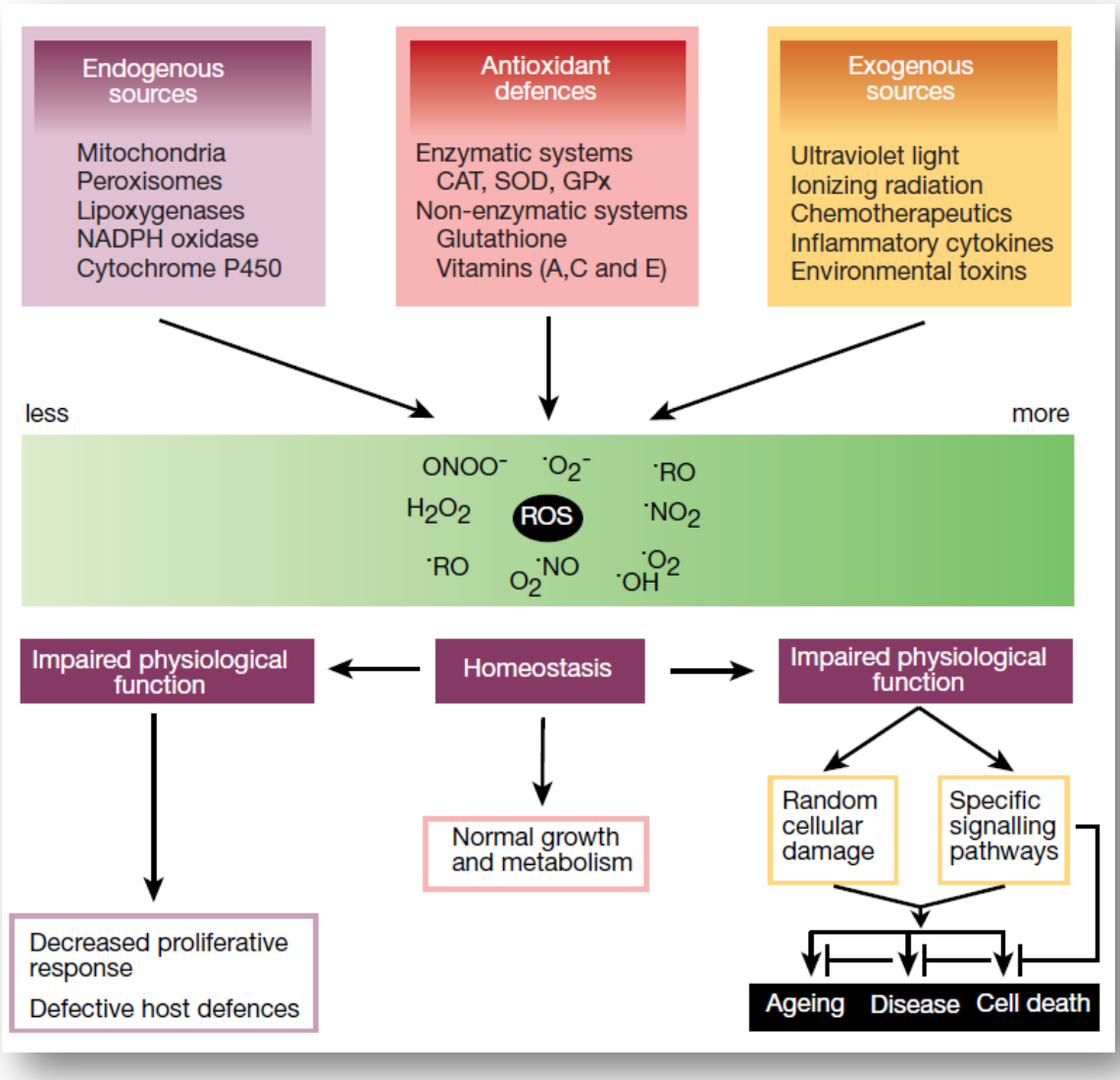

Figure 12 – Reactive oxygen species (Finkel/Holbrook)

As can be seen in the figure, there are a number of cytosolic enzyme systems that contribute to oxidative stress. Their relation to the purine degradation pathway will be discussed further later, in particular the role of NADPH oxidases (a superoxide-generating system that was first described in the neutrophil).[91]

1.3 Uric acid

Uric acid (also referred to interchangeably as urate) is the metabolic breakdown product of purine nucleotides, consisting of carbon, hydrogen, nitrogen and oxygen with the formula $C_5H_4N_4O_3$ and the structure shown in Figure 13.[98]

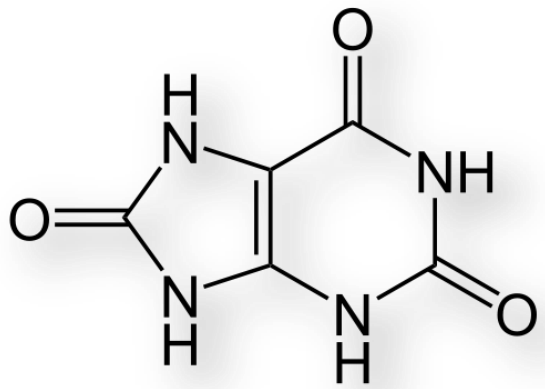

Figure 13 – Structure of uric acid

The most common medical implication of uric acid is the formation of monosodium urate crystals in joints leading to gout. Uric acid is produced mainly in the liver and to a lesser extent in the small intestine. Its production depends on the balance between purine ingestion, de-novo synthesis in cells, recycling, and the degradation function of xanthine oxidase at the distal end of the purine pathway (see Figure 16).[99]

Around 70% of excretion occurs via the kidneys therefore impairment of renal function is one possible cause for raised serum levels. Human beings and higher primates do not have the enzyme uricase that degrades uric acid to the highly soluble allantoin.[100] Therefore much higher urate levels are found in humans than are those of most non-primate mammals, fish, and amphibians that possess uricase. Consequently, the physiological concentration of urate in humans is close to its limit of solubility.[99] Thus

diuretic drugs (especially thiazides) may interfere with the renal excretion of urate, thus increasing urate levels and raising the chance of crystal formation – high intake of purine-rich foods may further compound matters.

Although it has an undisputed role in gout, uric acid has a more complex role when it comes to the cardiovascular system. There is consistent evidence of increased levels being associated with cardiovascular disease however this correlation has not always been independent of other risk factors.[101] Multiple studies have confirmed this association between baseline hyperuricaemia and cardiovascular disease, however a causal link has not clearly been proven.[102-105] Much of the data seems to demonstrate more of a linkage with other risk factors such as hypertension, dyslipidaemia and impaired glucose metabolism.[104]

However as mentioned in section 1.2, the uric acid molecule itself shows antioxidant behaviour – the majority of this role seems to be a scavenging ability with regards to peroxynitrite.[106] Peroxynitrite (formula $ONOO^-$) is formed by the reaction of nitric oxide (NO) with superoxide ($O_2^{\bullet -}$). The resulting molecule is itself not technically a free radical, as the unpaired electrons on the constituent superoxide and nitric oxide free radicals have each combined to form a new chemical bond, but it chemically remains a powerful oxidant that can damage a wide array of molecules in cells, including DNA and proteins.[107, 108] This appears to have particular relevance when considering the disease of multiple sclerosis (MS).

MS is a chronic T-cell mediated inflammatory demyelinating disease – as a result there has been much interest in the role that reactive oxygen and nitrogen species play in inflammation, demyelination and axonal injury.[109] There has been some interesting evidence to date of a positive role for uric acid in treating symptoms in animal models and of possibly stabilising lesion progression in humans through the use of inosine to raise uric acid levels.[110, 111] In addition, statistical evaluation of more than 20 million patient records for the incidence of MS and gout (hyperuricaemic) revealed that the two diseases are almost mutually exclusive, raising the possibility that hyperuricaemia may protect against MS.[106]

It is therefore unclear as to whether the increased levels of uric acid in diseases associated with oxidative stress are a cause of harm or instead a protective response. There is also the potential that uric acid may be neither good nor bad itself, but instead be an indicator of the activity of xanthine oxidase activity, itself an important source of reactive oxygen species, as will be discussed in the next section.[104]

There an interesting gender split with regards uric acid. Analysis of data from the Framingham study showed that with increasing age there was a minimal change in uric acid levels for men, yet a gradual increase from the fourth to seventh decade in women, as illustrated in Figure 14.[104] This may well be related to the role of oestrogen, which has a uricosuric effect.[112]

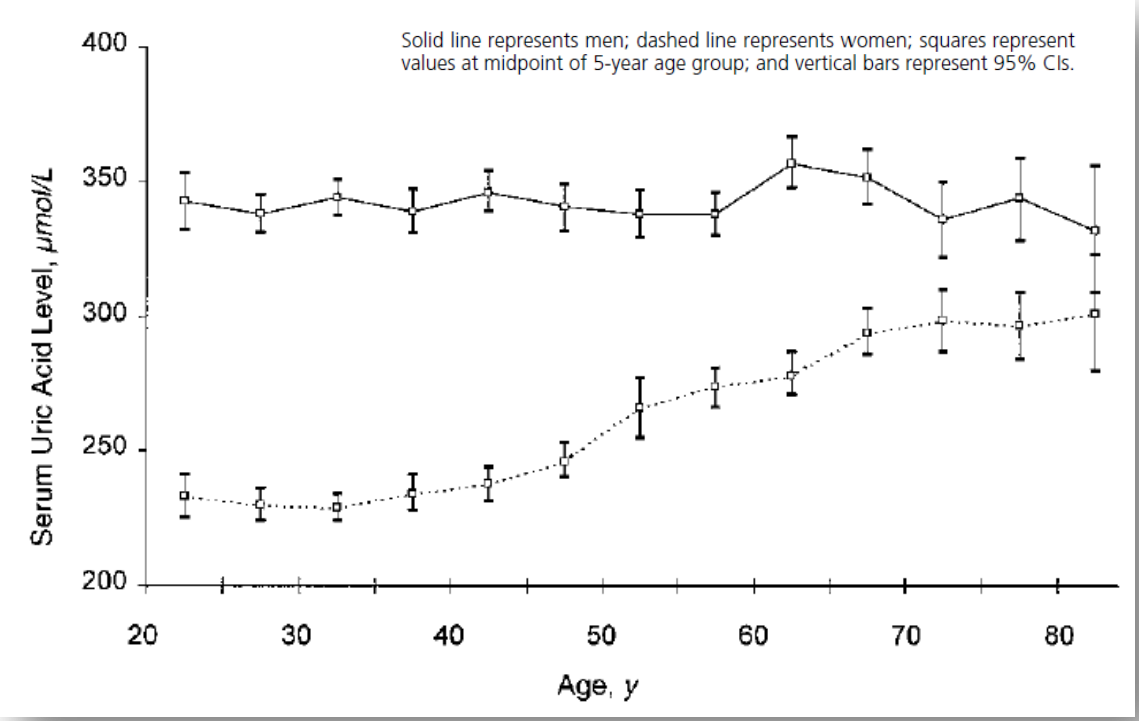

Figure 14 – Mean serum uric acid level by sex and 5-year age group

Most studies have not found a positive independent association between serum uric acid level and risk of cardiovascular disease in men. Those that have done so failed to account for confounders such as altered glucose metabolism, weight and diuretic use. In Framingham data this initially showed a strong and graded association between baseline uric acid and increased CV risk, but this risk was substantially reduced after adjusting for age and eliminated completely in a multivariate model.[104]

There has been some discussion around the potential role of uric acid in the metabolic syndrome. Potential mechanisms for this include the impact on endothelial function from hyperuricaemia (leading to reduced glucose uptake in skeletal muscle) and the inflammatory and oxidative changes uric acid appears to induce in adipocytes. In

hypertension animal models, hypertension appeared to be due to uric acid–mediated renal vasoconstriction resulting from a reduction in endothelial levels of nitric oxide, with activation of the renin–angiotensin system.[113] Consistent with these observations, elevated uric acid levels in humans also correlate with endothelial dysfunction and increases in plasma renin activity.[103]

In conclusion there is an association between uric acid and CV disease, however when properly analysed to take account of age, diuretic use and traditional CV risk factors this appears to be more a marker than a causative agent in and of itself. However the purine degradation pathway (which eventually produces uric acid) has a number of interesting components that probably play a role in oxidative stress and thus manipulation of the pathway may well be of therapeutic importance.

1.4 Allopurinol

1.4.1 History, pharmacokinetics, side-effects and alternatives

Given the association between uric acid and disease states as well as a potential role in oxidative stress there has understandably been great interest in whether reducing uric acid levels may have clinical utility. The main drug used to date for this role has been allopurinol.

As can be seen in Figure 15 below, allopurinol is a purine analogue (the similarities to uric acid can be seen by comparison to Figure 13) – a structural isomer of hypoxanthine and an inhibitor of xanthine oxidase, thus decreasing uric acid formation through the purine degradation pathway, as illustrated in Figure 16.[114, 115]

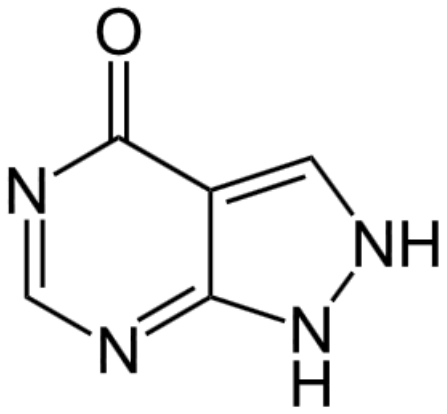

Figure 15 – Structure of allopurinol

Allopurinol was first synthesised in 1955 by Robins, who was investigating the possibility of developing new compounds that were isomeric with various biologically active purines in the hope that new anti-neoplastic agents might be discovered.[116] Although allopurinol itself was not anti-neoplastic, it was subsequently found by Elion

and Hitchings to inhibit the breakdown of one of the other purines, the chemotherapeutic agent mercaptopurine, leading to higher serum levels.[117] However it quickly became apparent that whilst allopurinol reduced the effective dose of mercaptopurine required in patients with chronic granulocytic leukaemia (in whom it was first tested), it did not appear to increase its therapeutic effectiveness. What was discovered though was that the hyperuricaemia and hyperuricosuria produced by tumour lysis syndrome aggressive chemotherapy in some patients with neoplastic disease could be averted and this new compound was then used almost exclusively for that purpose. [118-120]

However outwith oncology, allopurinol quickly became established in the main role it still occupies today – that of lowering uric acid levels to reduce the frequency of gout attacks.

Allopurinol interferes with the catabolism of purines by inhibiting the activity of the enzyme xanthine oxidoreductase (XOR). XOR is a complex molybdoflavoenzyme that, thanks to its ready availability in cows' milk (where it forms a major component of the milk fat globule membrane), has been known about for well over a century and in an essentially pure form for over 70 years. The cellular distribution of XOR is somewhat specialised, with relatively high concentrations being found in epithelial and endothelial cells. At first glance this would not seem consistent with its role solely as a housekeeping enzyme of purine catabolism and thus the relevance of it to other physiological functions has been sought – in particular the role it may play in ischaemia-reperfusion injury.[121]

XOR exists in two interconvertible forms in mammals, xanthine oxidase (XO) and xanthine dehydrogenase (XDH). It is found in various organs of the body although the highest levels of activity being found in the liver and intestine. Overall *in vivo* there is about an 80/20 split in favour of XDH, however due to endogenous serum proteases the majority of the XOR found in the circulation is in the XO form and thus commonly in the literature reference is made to XO rather than XOR.[121-124]

XO which has been released into the circulation has been observed to bind to a number of endothelia, including that of the vasculature. When bound to these endothelia it continues to exhibit normal reactivity and continues to produce superoxide, resulting in oxidative stress and endothelial dysfunction.[125] There is also some experimental evidence (albeit in mice) that nitric oxide inhibits XO activity.[126] Therefore in a situation where there is reduced NO, such as atherosclerosis, there may be increased XO activity and thus increased superoxide production which in turn then inhibits NO synthase, further decreasing NO bioactivity. Externally blocking this cycle via a XOR inhibitor may help break the downward spiral and help restore more normal endothelial function.

A small study by Spiekermann et al looked at both ex-vivo and in-vivo XO activity in patients with CAD compared to those without. The ex-vivo arm of the study (using post-mortem samples) demonstrated that in patients with CAD, XO and NADPH oxidase activity in the coronary arteries was increased compared with control subjects without atherosclerotic disease. In vivo, endothelial XO activity was increased in

patients with CAD compared with age-matched controls and found to be inversely related to endothelium-dependent vasodilation, suggesting that increased activity of XO was potentially contributing to endothelial dysfunction in patients with CAD. They found that XO-driven $O_2^{\bullet-}$ formation was abolished by oxypurinol, whereas NADP-mediated $O_2^{\bullet-}$ formation remained unaffected.[127]

The XO and XDH forms can be interconverted reversibly via sulphide reagents, or irreversibly (XDH to XO) by proteolysis. XO reduces only oxygen, whereas XDH is able to reduce either oxygen or NAD^+, but has a greater affinity for the latter.[128, 129]

Figure 16 provides a schematic representation of XOR-catalysed oxidation of xanthine and hypoxanthine at the molybdenum site, and of NADH, NAD, or molecular oxygen at the FAD site.[128] It can be seen that a by-product of the production of uric acid is the formation of ROS.[130]

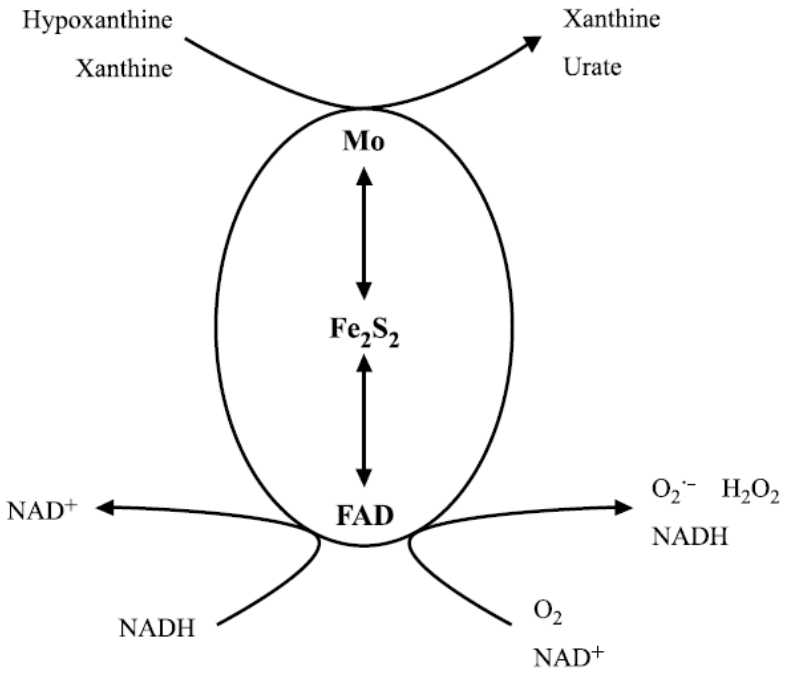

Figure 16 – Purine synthesis, salvation and degradation

As mentioned above, XOR was initially isolated from the bovine milk fat globule membrane. The enzyme can also be isolated from human breast milk, although due to its low molybdenum content the activity level is relatively low on the Mo binding sites. NADH activity on the other hand (which uses the FAD site) is very similar.[121]

As well as blocking XOR, allopurinol is also a substrate for it – the deriving metabolite, oxypurinol is a potent inhibitor of XOR itself and indeed may be considered responsible for much of the pharmacological effect.[131] The relative structures of allopurinol and oxypurinol are shown in Figure 17.[132]

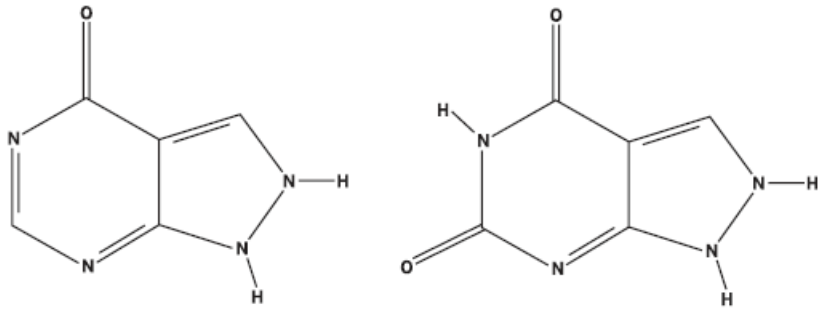

Figure 17 – Chemical structure of allopurinol and oxypurinol

Oral bioavailability of allopurinol is good (67-90%), with peak plasma concentration being reached within an hour. It is then rapidly metabolised in the liver by oxidation to oxypurinol within a few hours of ingestion. The mean elimination half-life of allopurinol is around an hour and oxypurinol around 18-40 hours. The renal clearance of both allopurinol and oxypurinol is lowered in elderly versus young subjects. [115, 132, 133]

Interestingly in studies of patients with xanthinuria (a rare genetic disorder where XOR deficiency leads to accumulation of xanthine) it appears that the enzyme aldehyde oxidase may also have a role to play in the conversion of allopurinol to oxypurinol.[134]

Allopurinol is generally well-tolerated as a drug, with the most frequently noted side-effects being gastrointestinal upset and skin rash. Other less common adverse effects include fever, jaundice, impaired renal function, leukopaenia, and thrombocytopaenia.

The more serious adverse effects tend to occur at higher doses and in patients with renal impairment. Neither allopurinol nor oxypurinol are significantly plasma protein bound, with both being renally excreted therefore it has been suggested that adverse effects of allopurinol are more frequent when there is renal insufficiency, however in clinical practice it appears that appropriate dose adjustment in patients with renal impairment is sufficient to mitigate against this being a major problem.[135, 136] There is some evidence that in patients where a reduced dose is insufficient to lower uric acid levels significantly then it is probably safe to cautiously proceed to higher doses.[137]

The most concerning potential side effect is that of Stevens-Johnson syndrome (SJS) as ultimately, in very rare cases, this may be fatal.[118, 138, 139] Allopurinol is the most common cause of SJS and toxic epidermal necrolysis in Europe.[140] However interestingly some recent research suggests that rather than this rare reaction being a dose-independent unpredictable event (what would conventionally be thought of as a 'type B reaction'), this may in fact be primarily driven by an oxypurinol-specific T cell response in a dose-dependent manner – something that has been found to be more marked in the presence of the HLA-B*58:01 allele.[132]

With regards to interaction with other drugs, the main concern is the reduction in metabolic clearance of mercaptopurine and azathioprine. There is some interaction with warfarin but with ongoing INR monitoring the anti-coagulant dosing is easily adjusted to take account of this. Overall clinical experience suggests patients tolerate the drug well, with only 1.8% of patients in a large drug monitoring study experiencing unintended/undesired effects.[118]

An alternative to allopurinol (currently only approved in the European Union) is febuxostat.[141] This is a novel non-purine xanthine oxidase inhibitor with approved doses of 80 mg per day and 120 mg per day. Dose adjustment is not necessary in patients with mild renal failure. Side-effects of febuxostat include raised liver enzyme activity and a small increase in the rate of serious cardiovascular events, in comparison to allopurinol, which therefore precludes its current use in patients with ischaemic or congestive heart failure.[99]

In a double-blind trial comparing febuxostat and a fixed dose of allopurinol (300mg per day) there was shown to be a greater reduction in uric acid levels with febuxostat but no significant difference in either gout flares or tophus area between the groups.[142]

Oxypurinol itself is available in some countries as a treatment option for gout, however given the excellent bioavailability of allopurinol, regardless of formulation, it has not gained widespread acceptance in that role.[100, 143, 144]

In section 1.2 the importance of reactive oxygen species was discussed. With regards to XOR, superoxide generation occurs in the presence of hypoxia when the enzyme transfers electrons to molecular oxygen rather than to NAD^+ during the formation of uric acid, thus contributing to oxidative stress.[101] It has been postulated that whilst this may implicate XOR to an extent in ischemia-reperfusion injury, it may also be the case that XOR-derived ROS and reactive nitrogen species (RNS) play a role in innate immunity, specifically in the inflammatory response and in anti-microbial defence of

the gastrointestinal tract – something that would fit with the levels of activity found in different tissues of the body.[121, 128]

1.4.2 Evidence of utility in cardiovascular disease

Given the role of XOR in superoxide formation there has been a great deal of interest in whether blockade of XOR activity via allopurinol is of clinical benefit.[130]

There is a logical potential pathophysiological mechanism for why this may be of benefit, particularly in ischaemic tissues. Firstly, molecular oxygen is a substrate for xanthine oxidase which consumes it and converts it into superoxide anions, hence depriving ischaemic tissue of vital molecular oxygen. Secondly, this "O_2 depriving" effect of XO is made worse by the fact that XO activity is upregulated in ischaemic tissue, hence initiating a vicious cycle whereby ischaemic tissue is deprived of oxygen even more.[145, 146]

The initial focus on oxygen was with regard to ischaemia-reperfusion injury, where oxygen is first switched off then suddenly available in abundance. In the early 1980s Granger *et al* first hypothesised a key role for XOR in this injury process. They postulated that during ischemia the energy status of the cell falls and transmembrane gradients break down. This leads to a rise in levels of intracellular calcium, which activates proteases that convert XDH, predominant *in vivo*, into XO.[147] Concomitantly, purines are catabolised and hypoxanthine accumulates. On reperfusion, oxygen is again available and, in the presence of XO and hypoxanthine, is reduced to hydrogen

peroxide and superoxide. These ROS interact to generate destructive agents such as hydroxyl anions and cause tissue damage.[148, 149]

Heart failure

At the turn of the millennium much of the research in the area of allopurinol and heart failure focussed on animal studies. There were promising results, both in rats and dogs, showing improved myocardial contractility (possibly in part due to a Ca^{2+}-sensitising property of XO inhibitors) and decreased myocardial oxygen consumption.[150-153] There was also a suggestion that both NOS and XOR signalling systems participate in the regulation of myocardial mechanical efficiency and that up-regulation of XOR relative to NOS contributes to mechano-energetic uncoupling in heart failure (particularly in the context of beta-adrenergic stimulation) and thus an opportunity for allopurinol to improve function.[154-156]

Research progressed to look at humans and Cappola *et al* found that intra-coronary injection of allopurinol in patients with idiopathic cardiomyopathy led to a 16% reduction (±5%, $p<0.01$) in myocardial oxygen consumption and an almost one-fifth improvement in myocardial efficiency.[157]

In 2002 Farquharson *et al* showed in a small randomised, placebo-controlled, double-blind crossover study that allopurinol improves endothelial dysfunction in chronic heart failure. This raised the possibility that allopurinol might reduce cardiovascular events and even improve exercise capacity in chronic heart failure.[158]

By the mid-2000s further clinical trials had been undertaken, the largest of which was the OPT-CHF study by Hare *et al*, looking at oxypurinol in patients with heart failure.[159-161]

The groundwork for OPT-CHF had been laid by the EXOTIC-EF study, which used intravenous oxypurinol.[162] It had shown a statistically significant improvement in left ventricular ejection fraction (LVEF) of almost a fifth, however it was a small open-label trial.

Around the same time the La Plata study reported. It was a month-long study of 60 patients with NYHA Class II-III heart failure, however by comparison it was a randomised, placebo-controlled double-blind trial. The main outcome of LVEF showed a 5% improvement in the active treatment group, although this did not reach statistical significance ($p<0.08$). Exclusion of those with an LVEF of >40% at baseline gave a statistically significant 7% increase in LVEF in the active arm ($p<0.02$), however it did reduce the numbers in the study by a third.[163] The efficacy of their dosing could also be questioned, with a reduction in uric acid level of only around 20%.

OPT-CHF by comparison was a much larger multicentre, randomised, double-blind, placebo-controlled, parallel group study of oxypurinol, this time involving just over 400 patients with a treatment period of six months. Uric acid reduction this time was marginally better at 25% in the active group however it did not produce a clinical improvement in the overall study population of patients with moderate-severe (NYHA III-IV) heart failure. In post-hoc analysis they did however find that those with higher

baseline uric acid levels responded better to oxypurinol both by way of percentage reduction in uric acid levels and also by way of clinical improvement. Again one does wonder whether the selected dose of oxypurinol was too low given that reductions in uric acid of almost double this level have previously been achieved using allopurinol.[164] Under-dosing is a common issue, with some studies looking for improvements in function after just a single dose rather than achieving a larger reduction in uric acid over a sustained period.[165] Importantly George *et al* (2006) showed that allopurinol had a steep dose-response curve in that 600mg of allopurinol produced twice the benefit of 300mg with regards endothelial function.[166] This again underscores the importance of getting the dose right.

Hypertension

As already mentioned in section 1.3, the Framingham Study provides useful information about uric acid levels in a large and well-monitored population. Sundström and colleagues made use of the data to investigate the relationship with hypertension.[167] They found that by the four-year follow-up point 13.8% of the 3,329 participants had developed hypertension and 36.1% had progressed to a higher stage of blood pressure. In multivariable analyses adjusting for age, sex, body mass index, diabetes, smoking, alcohol intake, serum creatinine, proteinuria, glomerular filtration rate, baseline BP, and interim weight change, a 1 SD higher serum UA was associated with an OR of 1.17 (95% CI 1.02 to 1.33) for developing hypertension, and an OR of 1.11 (95% C, 1.01 to 1.23) for BP progression. The serum uric acid level therefore appeared to be an independent predictor of hypertension incidence and BP progression in the study population.

In the same year Alper and colleagues published their analysis of the effect of uric acid levels in children and adolescents participating in the Bogalusa Heart Study.[168] They also carried out a multivariate regression analysis and found that elevated serum uric acid levels in childhood are associated with increased blood pressure both in childhood and higher blood pressure levels that persist into adulthood.

These data then naturally raised the question as to whether therapy aimed at lowering uric acid levels (by way of treatment with allopurinol) would be of benefit. Agarwal attempted to address this question in their meta-analysis of studies to date.[169] Unfortunately the small number of studies available in this area represented quite a heterogenous group, with widely varying dosage and inclusion/exclusion criteria. The ages of participants alone was varied, with mean ages ranging from 15 to 72 years.[170] The results did however show that there was a small, but statistically significant, reduction in both systolic (-3.3mmHg, 95% CI -5.3 to -1.4mmHg) and diastolic (-1.3mmHg, 95% CI -2.5 to 0.1mmHg) blood pressure readings. Some subsequent discussion of the meta-analysis challenged the lack of large RCTs and the strength of recommendation being offered, however the original authors did highlight that the role of allopurinol being envisaged was very much one of a later-stage agent and that further RCTs would be required prior to full recommendation in that regard – however in patients already at increased risk of gout (e.g., through use of diuretics), they felt it may be information that was of interest to clinicians when considering their prescribing strategy.[171, 172]

Endothelial function

As mentioned in section 1.2, the vascular endothelium is a complex structure that is vulnerable to damage from ROS, in particular through inactivation of NO.[173, 174] Given the mechanistic benefits allopurinol may be able to bring in reversing this trend and the previous *in vitro* studies showing positive effects, there have been a number of studies looking at the potential clinical benefits of this approach to improving endothelial function.[175, 176]

Initially hyperuricaemia itself was viewed as being a potential causative agent in vascular stress, in particular due to the impaired flow-mediated dilatation in hyperuricaemic patients.[177, 178]

However more recently there has been emerging evidence that the benefit of allopurinol is more through its effect on reducing vascular oxidative stress. George and colleagues showed this by comparing uric acid lowering by way of allopurinol compared to probenecid. Despite very similar reductions in uric acid levels (44% vs 46% respectively), there was no effect on endothelial-dependent vasodilatation in the probenecid group, compared to a 52% improvement in the allopurinol group.[166]

This positive effect of allopurinol independent of uric acid lowering has also been shown more recently by Jalal *et al*. They examined the data of over a hundred healthy volunteers participating in a community-based study in Boulder, Colorado, USA. They all had endothelial-dependent/independent dilatation examined by way of flow-mediated dilatation (FMD) and brachial artery response to GTN spray. They found that

individuals with the highest quartile of uric acid tended to be older and more likely to be male compared to those in the lowest quartile. They found no significant change in endothelial-dependent dilatation (via FMD) across increasing uric acid levels. They found a non-significant tendency for endothelial-independent dilatation (via GTN) to reduce slightly with increasing uric acid levels, along with a significant increase in CRP levels and a non-significant trend to increase in Oxidised LDL levels.[179]

Endothelial function has been assessed in a number of other co-existent disease processes. In hyperuricaemic patients with heart failure, XO inhibition with allopurinol was shown to improve peripheral vasodilator capacity and blood flow both locally and systemically.[180] It has also been shown to improve forearm blood-flow in patients with type II diabetes mellitus.[181] In patients with CAD both oxypurinol and allopurinol have been shown to be beneficial in improving measures of peripheral endothelial function.[182, 183] However there have been some slightly mixed reports when solely hypercholesterolaemia is considered, with Cardillo *et al* finding an improvement (albeit not normalisation) yet O'Driscoll *et al* finding no change.[184, 185]

What about patients without overt cardiovascular disease? Guthikonda and colleagues looked at a group that would be expected to have high levels of ROS-mediated endothelial damage – smokers. Their study was a randomised, crossover design with 14 smokers and 14 age/sex-matched non-smokers, all of whom had no other risk factors for atherosclerosis. They also only looked at a single-dose of allopurinol rather than long-term dosing and the study was only single-blinded. They did however find evidence of endothelial dysfunction, in that the responses to intra-

arterial bradykinin and acetylcholine (both endothelium-dependent dilators) were impaired in smokers compared to non-smokers. Administration of a single dose of allopurinol brought the response from smokers up to non-smoker levels with no change in the latter group. Their data suggested that stimulated NO production was reduced in smokers and improved by administration of allopurinol – it was theorised that this was most likely from reduced superoxide production due to XO blockade.[186]

Diabetes

Patients with diabetes have a much higher prevalence of LVH than non-diabetics, regardless of whether or not they have concomitant hypertension. Those with LVH and hypertension also have a blunted response to treatment with anti-hypertensive agents.[187]

There is evidence that treatment with allopurinol produces a reduction in LV mass in various animal models and in those with coronary artery and chronic kidney disease.[188, 189] It has recently also been shown to be beneficial in patients with diabetes mellitus, even although the magnitude of reduction was not as big as in anti-hypertensive trials this may still be beneficial in the long term. In other words, allopurinol acts on the BP-independent contribution to LV mass. This reduction in LV mass was thought to be due to the reduction in oxidative stress-mediated myocardial hypertrophy.[190]

There is also evidence of dysfunctional endothelial NO synthase function in patients with diabetes that led to increased vascular superoxide production.[191] It has been

shown that treatment with allopurinol in patients with diabetes has the ability to restore endothelial function in these situations.[181]

Ischaemic heart disease

As discussed above, there is evidence of the benefit of allopurinol in patients with IHD by way of LVH regression.[188] There is also evidence that even in patients already on contemporary, evidence-based treatment for IHD endogenous xanthine oxidase continues to generate vascular oxidative stress and widespread endothelial dysfunction. Treatment with allopurinol in these patients led to a significant improvement in acetylcholine-mediated vasodilatation on allopurinol compared with placebo, raising the prospect that high dose allopurinol (or its active metabolite, oxypurinol) might reduce future atherothrombotic events in CAD over and above the current therapies.[182, 183, 192]

However the question then arises – do these improvements in endothelial function and oxidative stress translate into clinical improvement for patients with angina? Noman *et al* showed that treatment with high-dose allopurinol significantly prolonged the time to ST segment depression, the total exercise time, and the time to angina in patients with chronic stable angina during a standard exercise test.[193] This suggests that endogenous xanthine oxidase activity contributes in some way to exercise-induced myocardial ischaemia. The previously discussed mechanisms of improved myocardial contractility and reduce oxygen consumption in the case of patients heart failure are additional mechanisms that are likely to contribute to its efficacy in IHD and

open up the potential of it being used as an add-on treatment to conventional therapy.[194, 195]

In addition, Rekhraj *et al* (2013) showed that allopurinol reduced LV end systolic volume and LV afterload (as measured by augmentation index) in patients with ischaemic heart disease, which suggests that offloading the LV may be another mechanism contributing to the anti-ischaemic effect of allopurinol in angina pectoris.[188] This possibility is highly relevant to the work in this thesis since this particular effect on LV afterload would produce an anti-ischaemic effect on the heart but not an anti-ischaemic effect in PAD.

1.5 Summary

Peripheral arterial disease is an important issue that affects a large number of patients with risks of significant morbidity and mortality. At present there are limited treatment options available to patients, particularly when compared to other comparable vascular diseases such as angina.

Smoking cessation and modification of risk factors remains the cornerstone of therapy in PAD. In those with severe symptoms intervention by way of angioplasty or surgery are viable options with varying success in individual patients. However the majority of patients are limited to medical therapy, which in itself mainly acts only to modify risk factors rather than treat symptoms. The small number of drugs are available to treat symptoms are expensive and have limited efficacy.

An inevitable consequence of normal intracellular metabolism is the production of reactive oxygen species. Of particular relevance here is the production of hydrogen peroxide and superoxide. This happens as a by-product of the formation of uric acid by XOR and thus blockade of this process may be beneficial in reducing the endothelial dysfunction caused by ROS.

Allopurinol is a well-established drug that is highly effective in blocking XOR and reducing uric acid levels. It is a mainstay of the preventive treatment of gout and has shown some promise in a number of cardiovascular conditions.

This cardiovascular improvement is likely to be through its effect on oxidative stress and NO in ischaemic tissue and an oxygen-sparing effect in tissues – there is therefore an important research question to be answered which is whether allopurinol will exert an anti-ischaemic effect in PAD. This is a key question given that allopurinol is anti-ischaemic in an analogous group of patients (those with angina pectoris).

2 Methods

2.1 Study design

This study was a randomised, double-blind parallel-group placebo-controlled clinical trial, run in accordance with the Declaration of Helsinki and The Medicines for Human Use (Clinical Trials) (Amendment) Regulations 2006.

The aim of the study was to examine whether high dose allopurinol prolongs exercise duration in patients with PAD.

2.2 Approvals

Sponsor approval

Reference: 2009CV16

Submitted: 9th April 2010

Sponsorship was confirmed and permission to apply for CTA granted on the 12th May 2010.

Ethical approval

Reference: 10/MRE00/51

Submitted: 31st May 2010

An initial favourable reply from Scotland A REC was received on the 28th June 2010 with minor amendments requested. Amendments were submitted on the 7th July 2010 and final approval received on the 19th July 2010.

NHS R&D approval

Reference: 2009CV16

Submitted: 9th July 2010

Approval was received on the 4th February 2011 (once MHRA approval had been received).

MHRA approval

Reference: 21726/0273/001-0001

Submitted: 17th June 2010

This took a number of months to process – the timetable of events was as described in Table 3 below.

Date	Progress
17th June 2010	Initial submission made.
13th August 2010	Initial MHRA reply – non-approval on pharmaceutical points, wished to know the amount of lactose in the placebo and how the placebo capsules were being tested to confirm the absence of allopurinol.
23rd August 2010	Reply sent to MHRA including detailed note from Tayside Pharmaceutical following their telephone discussions.
8th September 2010	Reply from MHRA – accept the changes but still wish to know the precise amount of lactose and require a test batch to be made to determine this.
18th November 2010	Test batch manufactured and revised IMP dossier sent to MHRA.
14th January 2011	Full MHRA approval received.

Table 3 – MHRA application timetable

Trial registries

ISRCTN: the study was registered and allocated reference ISRCTN01772998.

ClinicalTrials.gov: the study was registered and allocated reference NCT01147705.

EudraCT: the study was registered and allocated reference 2010-020662-23

Funding

An application for funding was made to the British Heart Foundation on 12th August 2009. This was considered by their Fellowships Committee and an award covering the full costs of the study was confirmed on 27th January 2010 – BHF Clinical Research Training Fellowship FS/10/014/28079.

The overall timeline for study setup is as show in Figure 18.

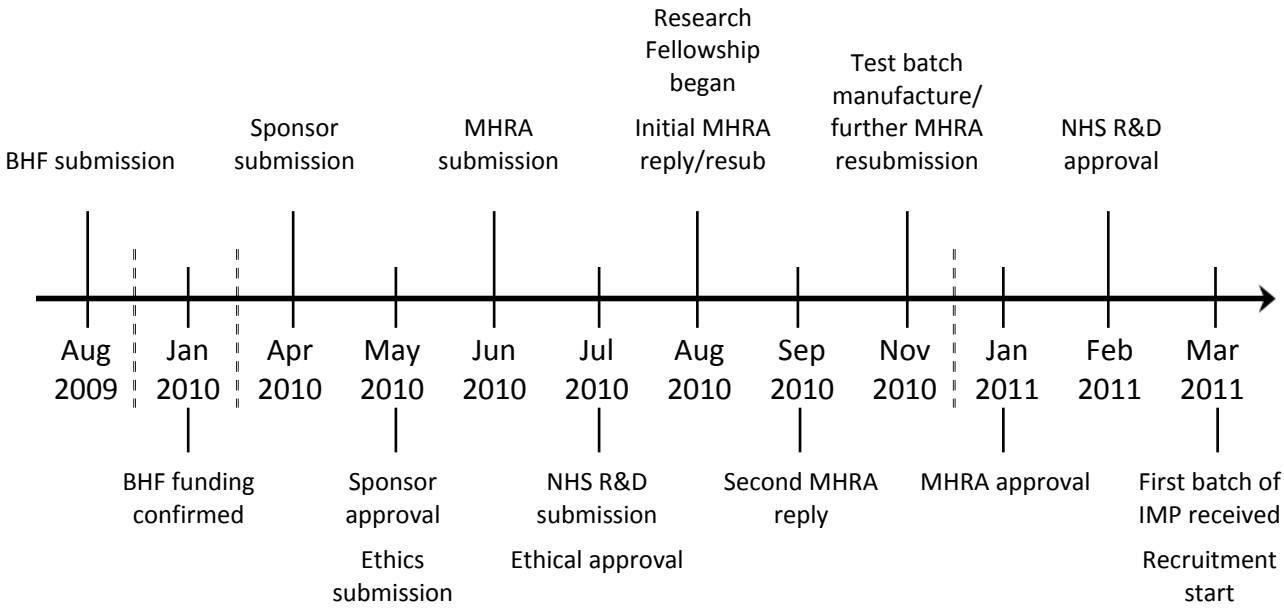

Figure 18 – Study timeline

2.3 Inclusion/exclusion criteria

The inclusion/exclusion criteria were as noted in Table 4 below.

Inclusion	• men and women age 35-85 years suffering from PAD
	• PAD defined as:
	  ○ claudication defined as leg pain on walking and disappearing within 10 minutes on standing and of presumed atherosclerotic origin *and*
	  ○ an ankle brachial pressure index (ABPI) of <0.90 on the worst leg at rest
	• stable disease by having a reproducible pain free walking distance on two consecutive treadmill tests, i.e., less than 25% variance
	• the reason for termination of the test must be claudication pain only

Exclusion	• rest pain • childbearing potential not taking adequate contraceptive measures • heart failure or any other exercise limiting cardiac disease • BP >180/100 mmHg • renal or liver disease • malignancy • already on allopurinol or had an adverse reaction to it • recent marked change in symptoms or recent (in the last six months) intervention for PAD • receiving treatment with either 6-mercaptopurine, azathioprine, warfarin, or theophylline

Table 4 – Inclusion/exclusion criteria

2.4 Cohort size & power calculation

A meta-analysis of exercise treatment trials in PAD and several drug trials was initially used to guide the required power for the study. In randomised trials in PAD, the SD tends to be around 30 metres.[49, 196-199] In drug treatment trials, the time to claudication usually increases by around 30 metres (on average 30% more than placebo).[49, 196-200] A 30 metre improvement is thought to make a clinically significant difference in PAD. If it is assumed a 30 metres improvement with a 30 metres SD, then only 42 subjects total in a parallel group study would be required to demonstrate a 30 metre improvement over placebo (for 90% power at $p<0.05$). However to allow for drop-outs, 50 PAD subjects were enrolled in this study.

2.5 Recruitment

2.5.1 Intermittent claudication clinic

With the exception of one patient (who directly approached AJR after seeing the trial listed on the ClinicalTrials.gov website), all patients in the study were recruited following their attendance at the Intermittent Claudication Clinic at Ninewells Hospital, Dundee. This clinic is run by a Vascular Specialist Nurse (Gill Crowe) – some patients were informed of the study by GC and their details passed to AJR to contact them with further information. However the majority came from AJR retrospectively going through clinic lists to pre-screen potential participants. Where patients met the inclusion/exclusion criteria on the basis of their clinic letter a covering letter was sent out by GC (in her capacity as the link clinician that patients had previously had contact with) enclosing an invitation from AJR, a Participant Information Sheet (PIS) and a (postage-paid) reply slip (Appendices A-C) – all approved by the Scotland REC A panel.

All those who replied indicating they would be interested in participating in the study were telephoned by AJR. Any missing information relevant to inclusion/exclusion criteria was checked in this phone call and then patients were offered an appointment for an initial screening visit.

2.5.2 Informed consent

All the participants had received the PIS at least 24 hours in advance of their attendance for a screening visit. The programme of study visits was outlined and all

had the opportunity to ask questions prior to completing a written consent form (see Appendix D).

2.6 Study visits

All study visits were conducted at the Department of Clinical Pharmacology, Ninewells Hospital, Dundee.

Following written informed consent the screening visit continued, with an initial clinical assessment comprising of:

- past medical history
- allergies & drug history
- family/social history
- physical examination
- height
- weight
- resting blood pressure
- measurement of ABIs

Following the above, the visit progressed to baseline exercise testing and phlebotomy. The tests involved are described in full in section 2.8. The subsequent study visits involved combinations of these tests, as noted in Table 5 below. Participants received oral and written confirmation of all study visits (see Appendix E for examples)

- Visit 1 (week 0) – screening visit 1
    - Participant consent – answer outstanding questions & complete consent form
    - Clinical assessment
    - Baseline ABI
    - Baseline ETT
    - Bloods for FBC/UE/LFT/uric acid/oxidised LDL
    - Record list of current medications

- Visit 2 (week 0) – screening visit 2
    - Second baseline ETT – if stable (<25% variance) then can continue in study
    - Six minute walk test
    - FMD measurement
    - Supply of initial study medication to participant along with instructions
    - Walking Impairment Questionnaire
    - Quality of life (SF-36 questionnaire)
    - Record list of current medications

- Visit 3 (week 6) – progress visit
    - Assess medication compliance
    - Check for AEs
    - Bloods for FBC/UE/LFT/oxidised LDL
    - Record list of current medications
    - Supply of full dose study medication (first half)

- Visit 4 (week 12) – progress visit
    - ETT
    - Six minute walk test
    - Assess medication compliance
    - Check for AEs
    - Walking Impairment Questionnaire
    - Quality of life (SF-36 questionnaire)
    - Record list of current medications
    - Supply of full dose study medication (second half)

- Visit 5 (week 18) – progress visit
    - FMD measurement
    - Assess medication compliance
    - Check for AEs
    - Bloods for FBC/UE/LFT
    - Record list of current medications

- Visit 6 (week 24) – final visit
    - FMD measurement
    - ETT
    - Six minute walk test
    - ABI measurement
    - Assess medication compliance
    - Check for AEs
    - Bloods for FBC/UE/LFT/uric acid/oxidised LDL
    - Walking Impairment Questionnaire
    - Quality of life (SF-36 questionnaire)
    - Record list of current medications

Table 5 – Study visit schedule

2.7 Randomisation & medication dosing

All patients providing consent to enter the study were issued with a sequential screening number. Those who remained eligible for inclusion in the study following their screening visits were issued with a sequential randomisation number and issued with the IMP matching that number.

Randomisation had been carried out by Tayside Pharmaceuticals (Ninewells Hospital, Dundee) as supplier of the IMP, prior to commencement of study visits. This was achieved through a computer generated randomisation system using five blocks of ten to ensure equal numbers between active and placebo groups. The randomisation key was held in sealed envelopes by both Tayside Pharmaceuticals, Ninewells Pharmacy (in case of any need for emergency unblinding) and for safe-keeping in a locked fireproof cabinet by the Asthma & Allergy Research Group, Ninewells.

Participants were randomised to receive either allopurinol or placebo for the six months of participation. The initial dose was 100mg once daily of allopurinol (or placebo) for two weeks, rising to 300mg once daily allopurinol (or placebo) for four weeks, then continuing at 300mg twice daily of allopurinol (or placebo) for the remainder of the study. Patients were provided with both verbal and written instructions regarding medication – the latter included a contact telephone number for AJR in case of any issues around tolerability (see Appendix F).

At time of randomisation and issuance of the initial medication supply a letter was sent to the participant's GP (see Appendix G) and a copy filed in their medical notes (along with a copy of their signed consent form).

Subsequent to completion of the trial all participants were formally thanked by letter. Following unblinding both participants and their GPs were informed of which group they had been in. A summary of the trial results was sent to all participants after analysis had been completed. Examples of these letters are shown in Appendix K.

2.8 Outcome measurements

2.8.1 Primary and secondary endpoints

The primary endpoint was the change in distance walked on an exercise tolerance test from baseline over a six month period. This comprised of both the claudication onset distance (COD – when the participant first noticed claudication pain) and the peak walking distance (PWD – when they could walk no further and had to terminate the treadmill test).

The overall secondary objectives were to (a) see if allopurinol improved the quality of life in patients with PAD and (b) to investigate the antioxidant effects of allopurinol in patients with PAD. To that end there were a number of secondary endpoints:

- Six minute walk test (6MWT)
- Walking Impairment Questionnaire (WIQ)
- Quality of Life (SF-36) questionnaire
- Measurement of FMD to assess endothelial dysfunction
- Measurement of oxidised LDL as a plasma marker of oxidative stress

2.8.2 Exercise tolerance test

These were conducted using the Gardner protocol, as is common in PAD studies testing efficacy of new treatments.[23, 201] The machine used was a GE Marquette Series 2000 Treadmill Stress Testing System (GE Healthcare Clinical Systems (UK) Ltd, Hatfield, UK). The tests were conducted in accordance with the University of Dundee SOP for ETTs and were supervised by AJR (PI and Advanced Life Support instructor) and

a second member of staff. Full resuscitation equipment (including external defibrillator) was available in the ETT room. Although the main outcome measure for this study was claudication distance, the ECG electrodes were connected as usual to provide cardiac rhythm monitoring and the ability to assess ST segments should a participant complain of chest discomfort.

Exercise testing became popular in the 1960s after Bruce and colleagues[202] developed graded exercise protocols for patients with cardiac disease. In PAD it was more popular to use a 'constant load' test at a defined grade (usually 1.5mph at 7.5% incline) until Gardner and colleagues in the early 1990s explored the use of a progressive exercise protocol.[201] They showed intra-class correlation coefficients of COD and PWD during tests were R = 0.53 and R = 0.55, respectively for a single-stage/constant load treadmill test. By comparison, the respective R values utilising the progressive protocol were R = 0.89 and R = 0.93. There is a great heterogeneity in the functional capability of patients with PAD, thus a progressive test allows for patients with a wide range of capabilities to be assessed. A single-stage protocol is too fast for many patients, but for others is insufficiently taxing. The 2% increase every two minutes with the Gardner protocol (at a constant speed of 2mph) ensures that most patients with PAD will become symptomatic during testing, whilst not proving too challenging in the early stages for those with more marked symptoms.

There is some evidence that sequential testing of untreated patients with the graded treadmill test is not associated with a temporal improvement in the initial or absolute claudication distances or times.[20, 23] This is potentially of benefit in clinical trials as

fewer participants are therefore required to elucidate any small but clinically important changes, however there is not unanimous agreement on this evidence, as will be covered further in the discussion section.

ABIs play an important role in the initial diagnosis of PAD[52, 73] and there is evidence that they have some role in follow-up – particularly in evaluating response to procedural intervention or restenosis.[203] However there is also evidence that non-procedural treatments may improve treadmill performance and functional status without a significant change in ABI.[204] Change in ABI was therefore not considered to be a key outcome measure. For the sake of completeness it is however included in section 3.9.

2.8.3 Six minute walk test

This was carried out as per American Thoracic Society guidelines, in a quiet corridor on a 30m track marked at 1m intervals.[205] Participants were given standard instructions asking them to walk from one end to the other of the track as many times as possible in six minutes, stopping to rest if required and then continuing when they felt able. Standard encouragement was given every minute. The total distance completed (to the nearest metre) was noted in the CRF. The test was carried out at baseline and repeated on visits four and six.

The 6MWT was originally developed in the 1980s as a useful alternative to the already established longer 12-minute walking test.[206] It has most commonly been used in patients with COPD – hence the guidelines from the American Thoracic Society.

However it has also been studied in PAD, where it was found to yield highly reliable measurements, both by way of high reliability coefficient, low coefficient of variation and in correlation with claudication distances on graded treadmill testing.[207]

2.8.4 Walking Impairment Questionnaire

This questionnaire is widely used in the evaluation of PAD patient symptoms. It was first developed by Regensteiner *et al* in 1990 and continues in widespread use today.[20, 208, 209] As well as being a short and straightforward questionnaire for patients to answer, it provides good correlation with patient performance on the treadmill, especially when stratifying them into high and low performers.[210-212] It has also been demonstrated that in patients with PAD, lower WIQ stair-climbing scores are associated with higher all-cause and cardiovascular mortality, independent of their ABI or other covariates.[213]

The questionnaire is designed to assess the day-to-day impact of intermittent claudication on patients. It evaluates the site of pain (calves vs buttocks) and the degree to which different activities trigger symptoms – considering distance, speed and climbing stairs. Given the heterogeneity of symptoms between patients it is crucial that all these domains are covered adequately, something that tends to be neglected in less condition-specific questionnaires. Each of the three domains has a number of questions to assess the degree of difficult the patient is experiencing. Answers to each question are scored on a graded scale from 0 to 4 (with 0 representing an inability to carry out the task and 4 representing no difficulty) – these

scores are then multiplied by a pre-specified weighting to provide summary scores for each domain and an overall score.

The questionnaire (see Appendix H) was issued to patients to complete at baseline, mid-point and end of study visits. It was carried out on arrival in the department (after walking from the front door of the hospital) to ensure they were well-rested prior to any exercise testing. It has been validated for self-administration, therefore participants completed it themselves, with the exception of any questions they wished to clarify.[214]

2.8.5 Quality of life (SF-36) Questionnaire

The SF-36 is a multi-purpose, short-form health survey consisting of 36 questions. It is a generic measure, rather than being targeted to any specific condition or age group. When scored it provides summary measures for functional health and well-being as well as a psychometrically-based physical and mental health summary.[215] It has been widely utilised in thousands of studies previously, allowing for comparisons between conditions. It was revised to v2 in 1996 to improve the instructions and layout to make it easier for patients to self-administer and to provide a subtler scale of responses rather than dichotomous replies.[216] There is some previous discussion of using this questionnaire in patients with PAD and evidence that in addition to providing information on general health it is capable of detecting changes following intervention, especially in the physical functioning domain.[217-219]

The questionnaire (see Appendix I) was issued to patients to complete at baseline, mid-point and end of study visits. Patients completed it themselves, with the exception of any questions they wished to clarify.

2.8.6 Flow-mediated dilatation

Flow-mediated dilatation is a non-invasive technique used to evaluate endothelial function. It involves measurement of both arterial blood flow and vessel diameter – in the case of our department the brachial artery is used for these measurements.

Following baseline measurements, a blood pressure cuff is inflated to supra-systolic pressures (200mmHg). Occlusion of the brachial artery causes the tissues distal to the occlusion to become ischaemic. Hypoxia, hypercapnoea and consequent decreasing pH lead to dilation of the vascular bed within the ischaemic area. Consequent loss of vascular peripheral resistance allows effective reperfusion as the occlusion is released. Blood then flows with high velocity into the tissues fed by the brachial artery on cuff deflation. The induced high-flow state through the brachial artery is called reactive hyperaemia and causes friction on the endothelium of the arterial wall. This "shear stress" friction has been proposed as the trigger for endothelial release of NO.[220]

The measurements are repeated following the administration of glyceryl trinitrate (GTN) spray. The GTN acts as an exogenous NO donor, allowing for examination of the endothelium-independent dilatation and thus demonstrating the maximum obtainable vasodilator response.

Corretti quotes 40-60 patients in a parallel-group design study as being sufficient to demonstrate significant improvement, therefore the sample size involved in this study was felt to be sufficient.[173]

Subsequent analysis of the recordings is then undertaken after the patient visit is complete. Blood flow was expressed as the velocity time integral (VTI) (the area under the blood velocity/time curve for a complete cardiac cycle). This was determined at baseline (mean of at least two measurements during the first minute of each study) and following cuff deflation. Baseline vessel diameter (mm) was defined as the mean of all measurements during the first minute of each study. Maximal dilatation after hyperaemia was expressed as a percentage change from the baseline diameter.[221]

Figure 19 provides an illustration of the setup used (courtesy Corretti *et al*.). [173] The diagram shows both upper and lower cuffs in place. In an actual study only one cuff is used, although either upper or lower placement is recognised as being valid. Custom and practice in our department was to opt for lower cuff placement. An adjustable jig was used to hold the probe in place and ensure stability of the ultrasound image throughout each FMD study.

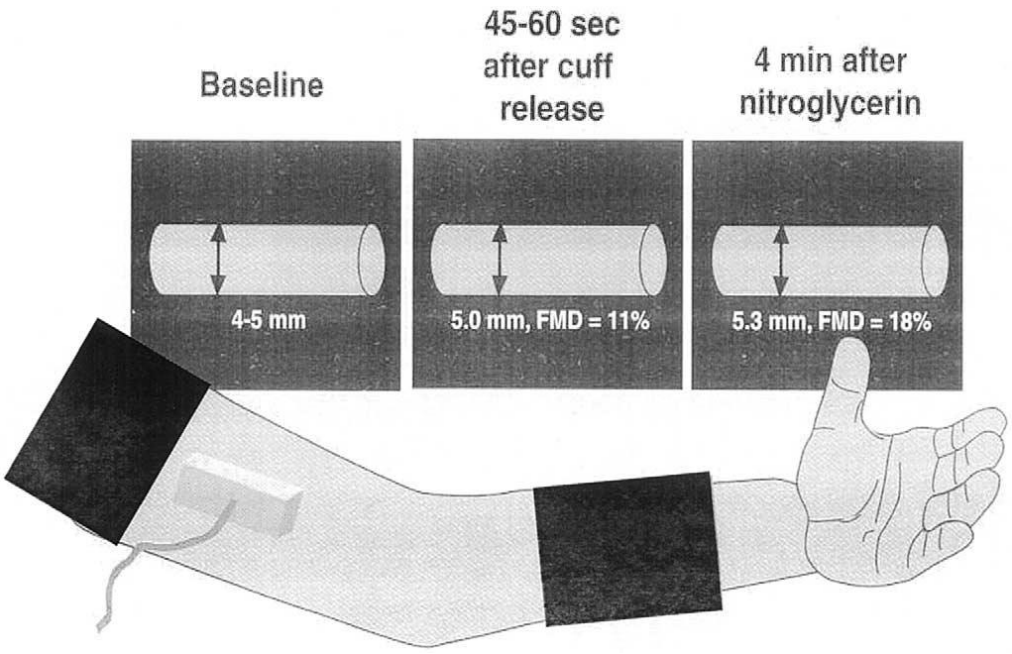

Figure 19 – Schematic drawing of brachial artery ultrasound imaging

FMD was performed at the baseline visit, at visit 5 (week 18) and at end-point (visit 6/week 24) using a Sequoia 512 (Siemens, Camberley, England) ultrasound machine with a 8 MHz linear array probe. The protocol used to carry out the measurements was the standard one used in the department, as displayed towards the end of the CRF in Appendix J. This is based on the International Brachial Artery Reactivity Task Force guidelines.[173] The FMD was analysed using Brachial Analyzer for Research, part of the MIA Vascular Research Tools software suite (v.5.6.12, Medical Imaging Applications LLC, Coralville, Iowa), again using our departmental SOP. The acquisition and analysis of the FMD images were performed by AJR, who was blinded to the allocated treatment.

2.8.7 Laboratory tests

A total volume of ~30ml venous blood was obtained on the initial visit. This was then split into sample tubes as follows: 5ml EDTA for Ox-LDL, 5ml serum for storage, 10ml plasma from the EDTA tube for storage, 5ml serum for biochemistry, 4ml EDTA for haematology. The haematology and biochemistry tubes were labelled with the participant's NHS details and hand-delivered to the NHS blood sciences laboratory on Level 7, Ninewells.

The study EDTA tubes were immediately put on ice and then centrifuged (along with the serum tube) for 10 minutes at 3,000rpm at 4^{o}C within 15 minutes of venepuncture. The EDTA plasma was extracted from the centrifuged samples and transferred into microtubes for analysis. These were individually labelled and then immediately stored at a -70^{o}C freezer until a later date (when assays were carried out in batches).
Venepuncture on subsequent visits was as described previously in Table 5.

Full blood count, urea & electrolytes/liver function tests/uric acid

As described above, all samples were hand delivered shortly after venepuncture to the NHS blood sciences laboratory (Ninewells Hospital, Dundee) where automated analysis was carried out to NHS Tayside standards. Results were received back the following day and after any uric acid results were removed from the sheets (via photocopying by LM with uric acid results covered up) they were recorded in the CRF by AJR. Any safety concerns arising from the routine haematology and biochemistry results were acted on appropriately by way of repeat sampling and where necessary IMP dose reduction.

In subsequent analysis the MDRD equation[222] was used to calculate eGFR based on creatinine in μmol/L:

$$\text{eGFR} = 32788 \times \text{Serum Creatinine}^{-1.154} \times \text{Age}^{-0.203} \times [1.212\ if\ Black] \times [0.742\ if\ Female]$$

The normal reference ranges for the NHS Tayside laboratory are as noted in Table 6. Partway through the study (February 2012) there was a major change in analyser equipment in the laboratory, from Roche to Siemens. This led to some minor adjustments in reference ranges and in the case of albumin, a change in methodology (also see comment in results, section 3.2).

Test	Normal reference range
Haemoglobin	13.0 – 18.0 g/dL (men), 12.0 – 16.0 g/dL (women)
White Cell Count	4.0 – 11.0 $x10^9$/L
Platelets	150 – 400 $x10^9$/L
Sodium	135 – 147 mmol/L
Potassium	3.5 – 5.0 mmol/L (Roche) 3.5 – 5.3 mmol/L (Siemens)
Urea	3.3 – 6.6 mmol/L (Roche) 2.5 – 7.8 mmol/L (Siemens)
Creatinine	62 – 106 µmol/L (men), 44 –80 µmol/L (women)
Alanine Transaminase (ALT)	13 – 43 U/L (Roche) 5 – 55 U/L (Siemens)
Alkaline Phosphatase (ALP)	40 – 130 U/L (Roche) 30 – 130 U/L (Siemens)
Bilirubin	0 – 17 µmol/L (Roche) 0 – 21 µmol/L (Siemens)
Albumin	36 – 50 g/L (Roche) 35 – 50 g/L (Siemens)

Table 6 – Normal reference ranges for NHS haematology and biochemistry tests

Oxidised LDL

Blood samples were obtained as noted above. Subsequent blinded analysis was carried out by the Vascular & Inflammatory Diseases Research Unit in Ninewells using Mercodia Oxidised-LDL ELISA. The background and rationale to selecting this test is discussed in full in section 4.2.6.

2.9 Data entry and management

Data from each study visit was recorded in the paper CRF designed by AJR prior to the start of recruitment (Appendix J). Treadmill printouts, blood results and questionnaire responses were all filed in the CRF (with the blood results sheets being suitably anonymised – the other items were labelled only by study ID). FMD data files were transferred by magneto-optical disk to the analysis computer for storage (and backup on an external drive) – following analysis the results were transcribed into the CRF.

Around the time of commencement of the study the Tayside Clinical Trials Unit had decided that electronic storage of study results should be carried out using the OpenClinica system. However as this study was one of the first 'live' projects to be put onto this system by the TCTU developers there were a number of delays in the system being available for use. As a result, data entry onto the system only finally started in the last month of the study and was essentially done retrospectively rather than as the study progressed (as had been originally envisaged). All CRF data was double-data entered – first by TCTU staff and then by AJR. All data entry and FMD analysis took place prior to unblinding – the database was locked and verified by TCTU staff following final data entry and secure copies were retained by TCTU prior to results being emailed to investigators and the trial statistician in the form of SPSS files.

2.10 Statistical analysis

Analysis was undertaken by AJR using IBM SPSS Statistics version 20 (IBM Corp, NY, USA), with feedback obtained from LW on the initial analyses to confirm the correct procedures were being followed. Following conclusion of the final trial visit (whilst data entry was still taking place) the copies of the allocation of treatment code held by both the NHS Tayside Pharmacy and the Asthma & Allergy Research Group were forwarded by the respective departments direct to TCTU. Only after completion of data entry, when TCTU had carried out database verification and lock and had distributed results to the trial statistician were the codes made available to AJR.

Data were checked for normality using the Shapiro-Wilk test given the smaller sample sizes involved in this study. Where the significance value of the Shapiro-Wilk test was below 0.05 the data was assumed to be non-normally distributed. In these situations it was log-transformed to see if this led to a normal distribution, thus allowing parametric analysis to be carried out. If it remained non-normally distributed then non-parametric analysis was used. Pearson's chi-squared was used for discrete variables. An independent samples t-test was used for normally-distributed continuous variables and a Mann-Whitney U test for non-parametric data. For repeated comparisons within the same subject a paired t-test was used for normally-distributed data and a Wilcoxon Signed-Rank matched pairs test for non-parametric data. The study statistician (LW) was in agreement with this protocol.

2.11 Adverse events

All adverse events were reported in accordance with The Medicines for Human Use (Clinical Trials) Amendment Regulations 2006. At each study visit participants were asked about any adverse events since their last study visit, including any hospitalisation, unscheduled GP visits or new disabilities. The sponsor was notified if there was a serious adverse event (SAE) or serious adverse reaction (SAR) except if it was a known adverse drug reaction that was set out in the Summary of Product Characteristics for allopurinol.

A record was kept of all changes to the patients routinely prescribed medications throughout the study, as well as any acute prescriptions issued by their General Practitioner.

3 Results

3.1 Recruitment

As noted in section 2.5.1, with the exception of one patient (who directly approached AJR after seeing the trial listed on the ClinicalTrials.gov website) all patients in the study were recruited following their attendance at the Intermittent Claudication Clinic at Ninewells Hospital, Dundee. Clinic lists from October 2009 to July 2011 were obtained and from these potential participants were identified. The clinic consists of a mix of patients with potential/known PAD and those with wound care issues; however the groups were clearly distinguished on the clinic lists allowing attention to be focused on those attending due to probable/known PAD – a total of 673 potential participants. Clinic letters for those patients were then screened to assess for potential eligibility to participate in the study – 519 were excluded at this stage due to failure to meet inclusion/exclusion criteria. The remaining 154 received written invitations as previously described in section 2.5.1.

The response rate from patients was excellent, with 74% (114/154) replying to the invitation letter. Those who had indicated a willingness to be contacted further (73) were telephoned by AJR. Of these, 66 attended for an initial screening visit – 16 failed screening, for the reasons noted in Figure 20 below. The remaining 50 were randomised into the study. This high conversion rate from screening to randomisation (76% - 50/66) was greatly aided by the high quality and thoroughness of the clinic letters that GC had written, which enabled early exclusion of those participants not meeting study criteria.

Following full NHS R&D approval in February 2011, full IMP manufacture was authorised. This was delivered in mid-March – participant recruitment was only able to start when a confirmed delivery date for this had been received, therefore the first screening visit did not take place until early April. The first ten participants had been randomised by the end of June 2011. Unfortunately recruitment had to be put on hold at the start of September 2011 as there had been no further IMP delivery from Tayside Pharmaceuticals (due to issues at their end in receiving the allopurinol bulk power from their supplier). Recruitment restarted after a three-week delay and the final screening visit was in early January 2012 with the last follow-up visit in June 2012.

Withdrawals during the study itself were low – 5 in total (10%) – these were evenly split between active and placebo groups and reasons for withdrawal are as noted in the CONSORT diagram in Figure 20 below.

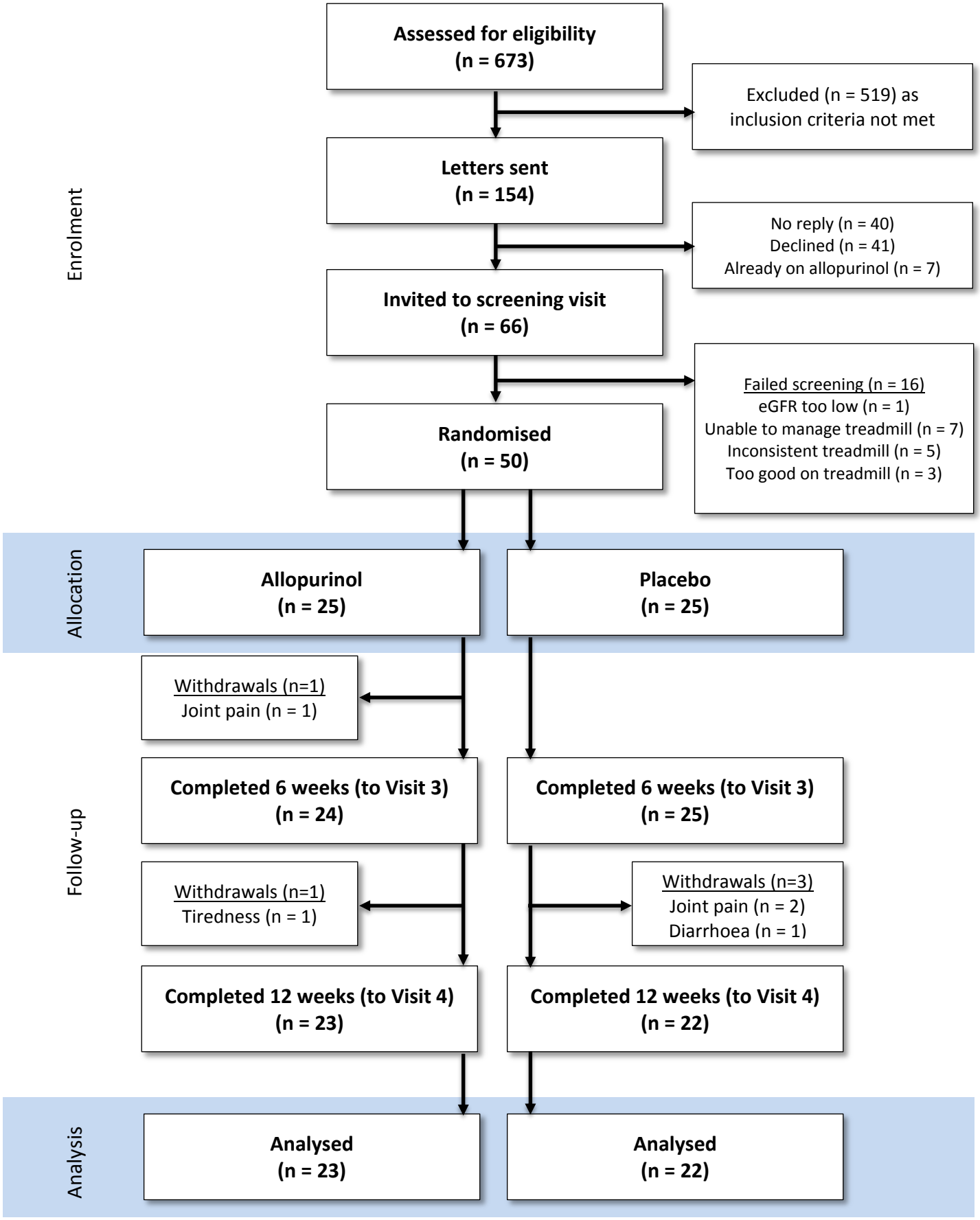

Figure 20 – CONSORT diagram

3.2 Baseline characteristics

Of the 50 patients randomised, 78% (39/50) were male. Participants had a mean (SD) age of 68.4 (1.2) years. The baseline demographics were as outlined in Table 7 below.

	Allopurinol (n = 25)	**Placebo (n = 25)**	**p**
Mean age (years)	69.6 (9.1)	67.3 (7.5)	0.34
Male sex	21 (84%)	18 (72%)	0.50
Height (m)	1.69 (0.09)	1.66 (0.07)	0.28
Weight (kg)	77.7 (16.6)	80.6 (16.6)	0.36
BMI (kg/m^2)	27.2 (5.1)	29.1 (5.2)	0.21
Pulse (bpm)	76.5 (11.6)	75.4 (14.9)	0.62
Pack years	31.7 (21.0)	49.7 (37.0)	**0.04**
Systolic BP (mmHg)	153 (21)	156 (20)	0.65
Diastolic BP (mmHg)	76 (11)	79 (10)	0.27
ABI	0.61 (0.12)	0.60 (0.12)	0.65
Average weekly alcohol intake (units/week)	7.4 (10.0)	16.8 (18.8)	0.08
Smoking status			
Current smoker	6	7	0.75
Ex-/non-smoker *	19	18	

Table 7 – Baseline characteristics of participants

Data displayed as mean (SD). Pearson's chi-squared was used for discrete variables, independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

* There were only two non-smokers in the study, both in the allopurinol group – to run a valid chi-squared test these there were combined with the non-smoker group.

	Allopurinol (n = 25)	Placebo (n = 25)	p	p
Concomitant medications				
Aspirin/Clopidogrel	24 (96%)	23 (92%)	0.88	
Beta-blocker	5 (20%)	9 (36%)	0.29	
Statin	23 (92%)	24 (96%)	0.88	
ACEi/ARB	17 (68%)	18 (72%)	0.87	
PPI/H2-antagonist	9 (36%)	5 (20%)	0.29	0.94 *
Long-acting nitrate	1 (4%)	1 (4%)	1.00	
GTN spray	6 (24%)	3 (12%)	0.32	
Painkiller	6 (24%)	6 (24%)	1.00	
Calcium-channel blocker	13 (52%)	9 (36%)	0.39	
Diuretic	7 (28%)	4 (16%)	0.37	
Naftidrofuryl	2 (8%)	1 (4%)	0.56	
Past Medical History				
Ischaemic heart disease	13 (52%)	10 (40%)	0.53	
Hypercholesterolaemia	6 (24%)	6 (24%)	1.00	
Hypertension	14 (56%)	14 (56%)	1.00	
COPD	2 (8%)	4 (16%)	0.41	0.98 *
Diabetes mellitus	2 (8%)	2 (8%)	1.00	
GORD	5 (20%)	3 (12%)	0.48	
Vascular surg/intervention	3 (12%)	3 (12%)	1.00	
Other	17 (68%)	19 (76%)	0.74	

Table 8 – Concomitant medications and past medical history

* Advice was taken from the study statistician (LW) with regard to calculated p-values for comparing both these distributions. If done line-by-line (see previous column) then a significant number of cells in the chi-squared test have a count of <5 – it was therefore suggested that an overall analysis of distribution be made, as shown in this column.

Baseline blood results comparing allopurinol versus placebo groups are as outlined below in Table 9.

	Allopurinol (n = 25)	**Placebo (n = 25)**	**p**
Sodium (mmol/l)	141.1 (2.7)	140.5 (3.4)	0.59
Potassium (mmol/l)	4.3 (0.2)	4.5 (0.3)	0.05
Urea (mmol/l)	6.1 (1.9)	5.8 (1.8)	0.62
Creatinine (mmol/l)	92.8 (18.5)	87.9 (21.6)	0.30
GFR (ml/min)	74.3 (17.1)	78.2 (20.0)	0.53
Haemoglobin (g/dl)	14.7 (1.4)	14.7 (1.1)	0.90
WCC ($x10^9$/l)	8.1 (2.2)	8.0 (2.1)	0.91
Neutrophils ($x10^9$/l)	4.7 (1.9)	4.9 (1.6)	0.70
Platelets ($x10^9$/l)	254.8 (57.0)	242.2 (53.2)	0.42
ALT (U/l)	23.5 (11.6)	33.1 (21.3)	0.08
Bilirubin (µmol/l) *	9.1 (3.5)	9.0 (5.0)	0.62
Alk Phos (U/l)	74.2 (18.4)	84.1 (35.9)	0.50
Albumin (g/l)	47.9 (2.7)	47.2 (2.4)	0.37
Oxidised LDL (U/l)	53.0 (17.8)	49.4 (15.3)	0.62
Uric acid (mmol/l)	0.36 (0.09)	0.34 (0.09)	0.98

Table 9 – Baseline blood results of participants

Data displayed as mean (SD). Pearson's chi-squared was used for discrete variables, independent samples t-test for normally-distributed continuous variables and Mann-Whitney U test for non-parametric data.

* Values <5 have been assumed to be equal to 5 for the purposes of statistical analysis

Safety blood results comparing initial and final visit results split into allopurinol and placebo groups are as outlined below in Table 10.

	Allopurinol Visit 1 (n = 25)	**Allopurinol Visit 6 (n=23)**	**p**	**Placebo Visit 1 (n = 25)**	**Placebo Visit 6 (n = 22)**	**p**
Sodium (mmol/L)	141.1 (2.7)	141.0 (2.2)	0.43	140.5 (3.4)	140.2 (3.5)	0.48
Potassium (mmol/L)	4.3 (0.2)	4.4 (0.2)	0.68	4.5 (0.3)	4.5 (0.3)	0.47
Urea (mmol/L)	6.1 (1.9)	6.3 (1.4)	0.79	5.8 (1.8)	5.8 (1.8)	0.97
Creatinine (mmol/L)	92.8 (18.5)	86.8 (19.5)	0.29	87.9 (21.6)	83.9 (21.5)	0.44
GFR (ml/min)	74.3 (17.1)	81.3 (24.5)	0.30	78.2 (20.0)	83.2 (22.0)	0.50
Haemoglobin (g/dL)	14.7 (1.4)	14.5 (1.3)	0.92	14.7 (1.1)	14.9 (1.3)	0.36
WCC ($x10^9$/L)	8.1 (2.2)	8.6 (1.9)	0.48	8.0 (2.1)	7.1 (1.4)	0.14
Neutrophils ($x10^9$/L)	4.7 (1.9)	5.2 (1.7)	0.18	4.9 (1.6)	4.2 (1.2)	0.22
Platelets ($x10^9$/L)	254.8 (57.0)	277.4 (60.2)	0.15	242.2 (53.2)	247.0 (62.7)	0.84
ALT (U/L)	23.5 (11.6)	29.1 (14.6)	0.15	33.1 (21.3)	29.8 (13.0)	0.99
Bilirubin (µmol/L) *	9.1 (3.5)	9.4 (2.7)	0.21	9.0 (5.0)	9.8 (4.9)	0.55
Alk Phos (U/L)	74.2 (18.4)	82.7 (21.7)	0.11	84.1 (35.9)	88.2 (45.3)	0.83
Albumin (g/L)	47.9 (2.7)	44.3 (4.0)	**0.002**	47.2 (2.4)	44.2 (3.9)	**0.005**

Table 10 – Safety blood results comparing initial and final visits

Data displayed as mean (SD). Pearson's chi-squared was used for discrete variables, paired t-test for normally-distributed continuous variables and Wilcoxon Signed Rank test for non-parametric data.

* Values <5 have been assumed to be equal to 5 for the purposes of statistical analysis

With regards the albumin results it can be seen there was a statistically significant reduction in both groups between the start and end of the study. This has been discussed with Dr Ellie Dow, Consultant in Biochemical Medicine in the NHS Tayside

laboratory used for the study and it was felt these reductions were most likely due to a change in methodology used in the laboratory. The original method (at the time of V1 sampling) made use of bromocresol purple, but during the course of the study the laboratory switched to using bromocresol green, which is felt to be closer to gold standard and actually more accurate at the lower end.

3.3 Adherence to medication

Uric acid levels were checked on both initial and final visits, giving a good idea of concordance with treatment. As noted in Table 9 there was no significant difference in baseline uric acid levels between the two groups (p=0.98). It can be seen in Figure 21 below that there was a highly significant drop in uric acid for those on treatment, with an order of magnitude of 52.1% (p<0.001). A small 10.0% drop was noted in the placebo group but this was not statistically significant (p=0.06). Two participants had either no change or an increase in uric acid level between start and end of the study, suggesting at least a period of non-compliance. All other participants in the active treatment arm had >13% reduction, with the majority a 40-60% reduction as indicated by the aforementioned mean.

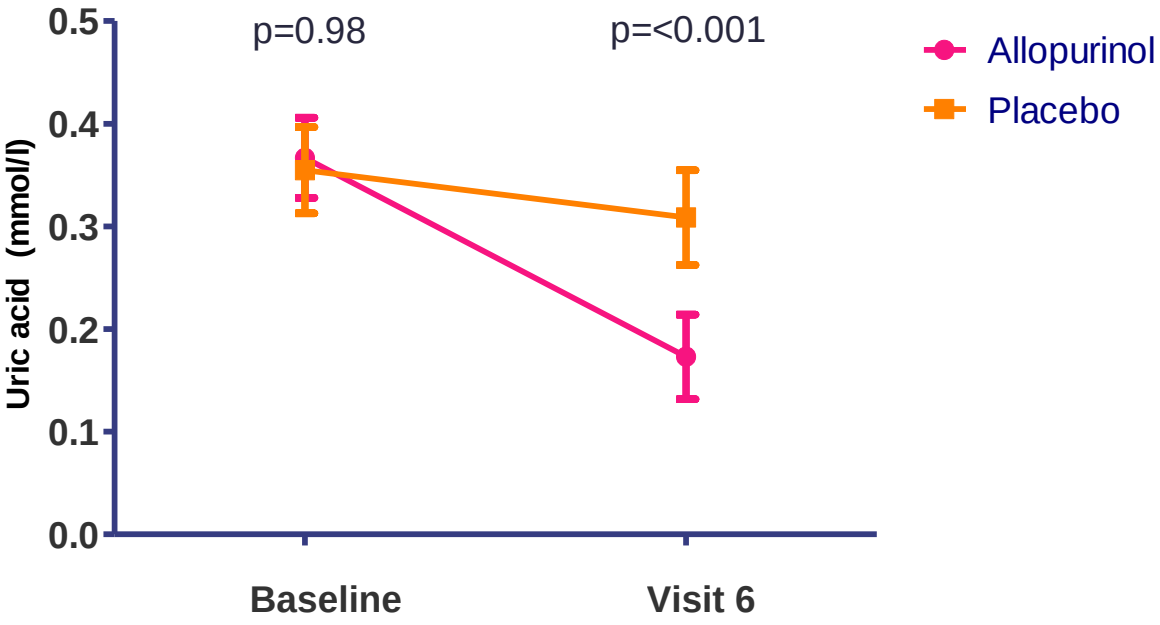

Figure 21 – Reduction in uric acid (vertical bars indicate 95% CI)

3.4 Adverse events

A total of 16 adverse events were reported in total, eight in each group. Table 11 summarises the reasons for these events. One patient had three adverse events and one had two, thus a total of 13 patients reported adverse events. Nine of the events were felt to be unrelated to the study drug. Of the other seven events the IMP was stopped in three cases and the dose reduced in a further three (UEs were rechecked in the remaining case).

One participant experienced a serious adverse event during the study, suffering a community acquired pneumonia that required hospital admission. This ended up being a prolonged admission and following discharge home they elected to withdraw from the study.

	Allopurinol	**Placebo**
Chest discomfort	1	
Chest infection		2
Diarrhoea		1
Dizziness	1	
Hospitalisation **(SAE)**	1	
Hyponatraemia		1
Joint pain	2	3
Minor deterioration in UEs	1	
Raised ALT		1
SOB	1	
Soft tissue infection	1	

Table 11 – Adverse events

3.5 Treadmill

The output from OpenClinica provided a time in seconds for each treadmill test for both onset of claudication and peak walking time. Given the constant 2mph speed of the treadmill throughout the test it was possible to convert this into a more clinically meaningful distance in metres (time in seconds x 0.89408ms^{-1}).

The change in claudication onset and peak walking distance was then analysed, comparing those on allopurinol with those on placebo. The natural logs of the distances involved were used to provide normal distribution for the data prior to either independent (between treatment group) or paired (within treatment group) t-tests being carried out.

The data was then sub-divided by a variety of factors:

- Factor: above/below median baseline claudication onset (section 3.5.2)
- Factor: above/below median baseline peak walking distance (section 3.5.3)
- Factor: above/below median baseline uric acid (section 3.5.4)
- Factor: above/below median baseline systolic BP (section 3.5.5)
- Factor: above/below median baseline ABI (section 3.5.6)

3.5.1 Without any sub-division by factor

Table 12 below shows the claudication onset distances for each treatment arm at different stages of the study. The baseline figure is the mean of Visit 1 and Visit 2 as was pre-specified in the study protocol.

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline (metres)	137.1 (82.0 to 192.2)	151.7 (105.4 to 198.0)	0.31
Visit 4 (metres)	161.7 (109.0 to 214.3)	155.0 (110.2 to 200.0)	0.95
Visit 6 (metres)	180.0 (111.5 to 248.6)	177.1 (133.1 to 221.2)	0.48

Table 12 – COD in metres at different study stages

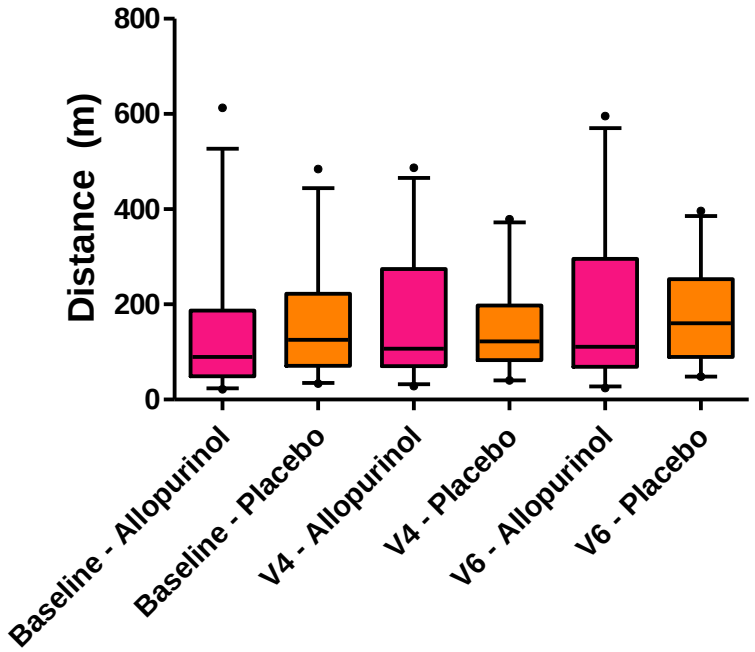

Figure 22 – COD in metres at different study stages

The change in claudication onset distance from baseline is shown in Table 13 and Figure 23.

	Allopurinol (95% CI)	**Placebo (95% CI)**	**P**
Mean change from Baseline to Visit 4 (metres)	19.4 (-11.1 to 49.8)	25.0 (8.3 to 41.6)	0.82
Mean change from Baseline to Visit 6 (metres)	37.7 (3.7 to 71.8)	47.1 (19.9 to 74.3)	0.35

Table 13 – Absolute change in COD from baseline

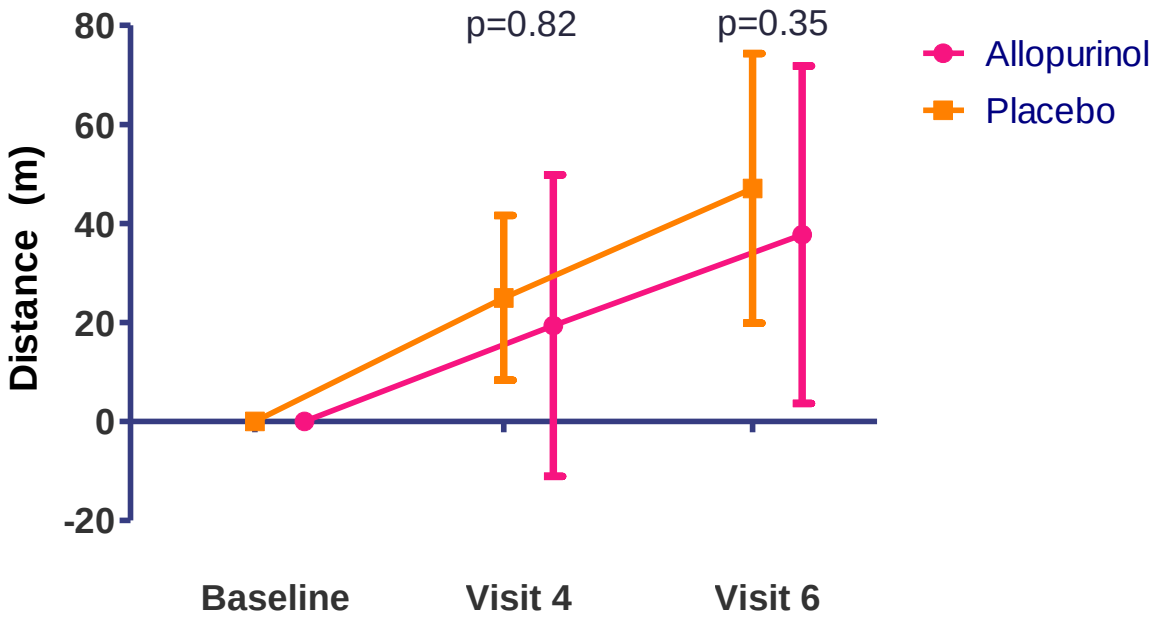

Figure 23 – Mean absolute change in COD (vertical bars indicate 95% CI)

Given the variability in baseline walking distances noted in Table 12, the mean relative change was calculated and is shown in Table 14 and Figure 24 below.

	Allopurinol (95% CI)	**Placebo (95% CI)**	**P**
Relative increase from Baseline to Visit 4	0.34 (0.14 to 0.54)	0.23 (0.10 to 0.37)	0.52
Relative increase from Baseline to Visit 6	0.41 (0.11 to 0.70)	0.50 (0.26 to 0.75)	0.47

Table 14 – Relative increase in COD from baseline

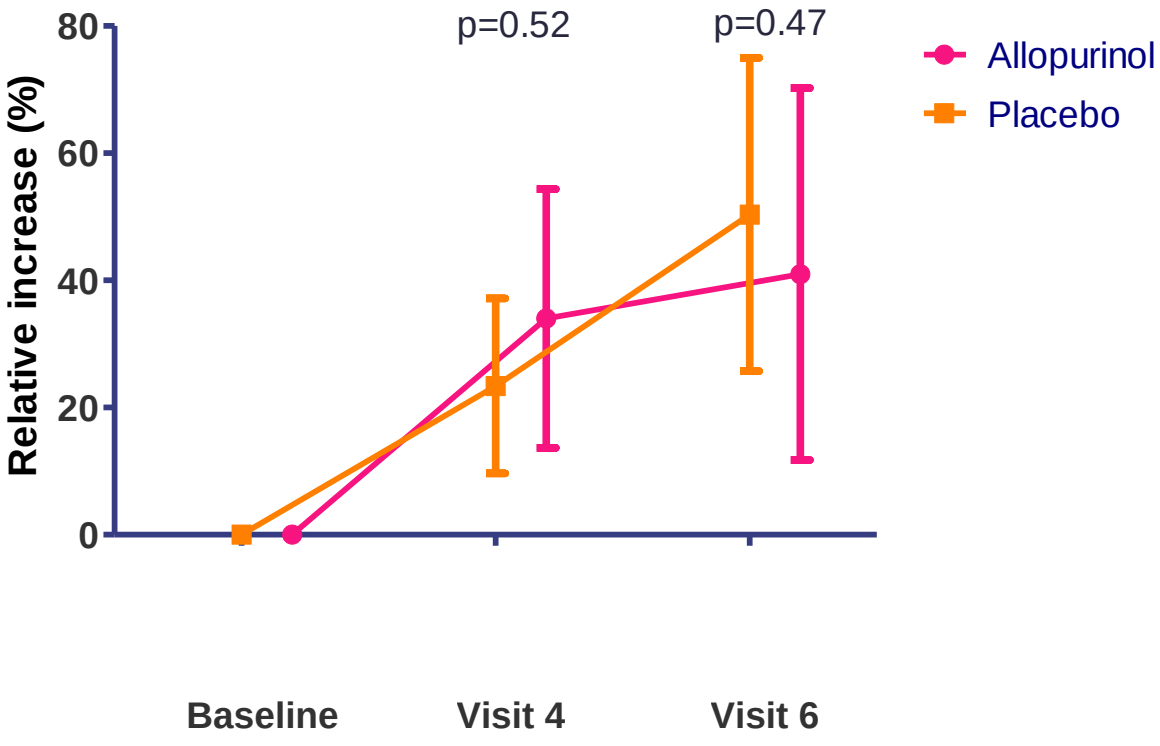

Figure 24 – Relative increase in COD (vertical bars indicate 95% CI)

The individual change for each participant is shown in Figure 25 and Figure 26 below.

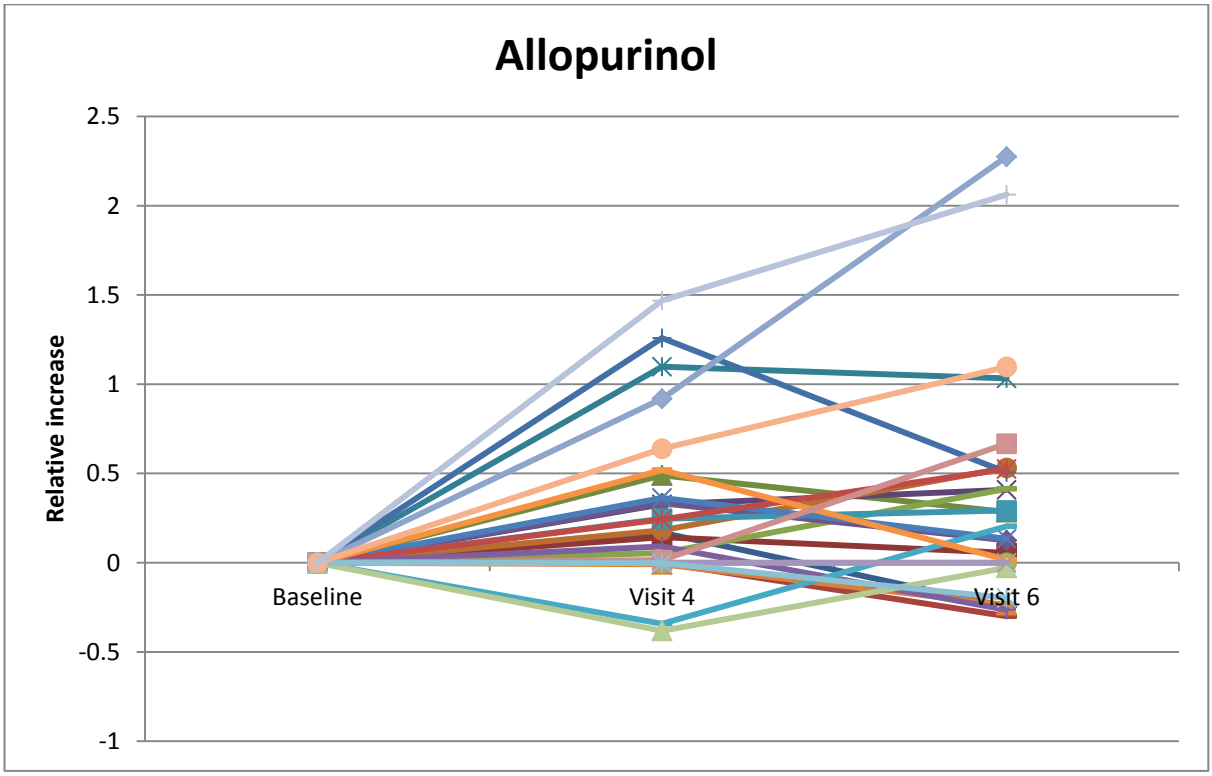

Figure 25 – Relative increase in COD (individual participants on allopurinol)

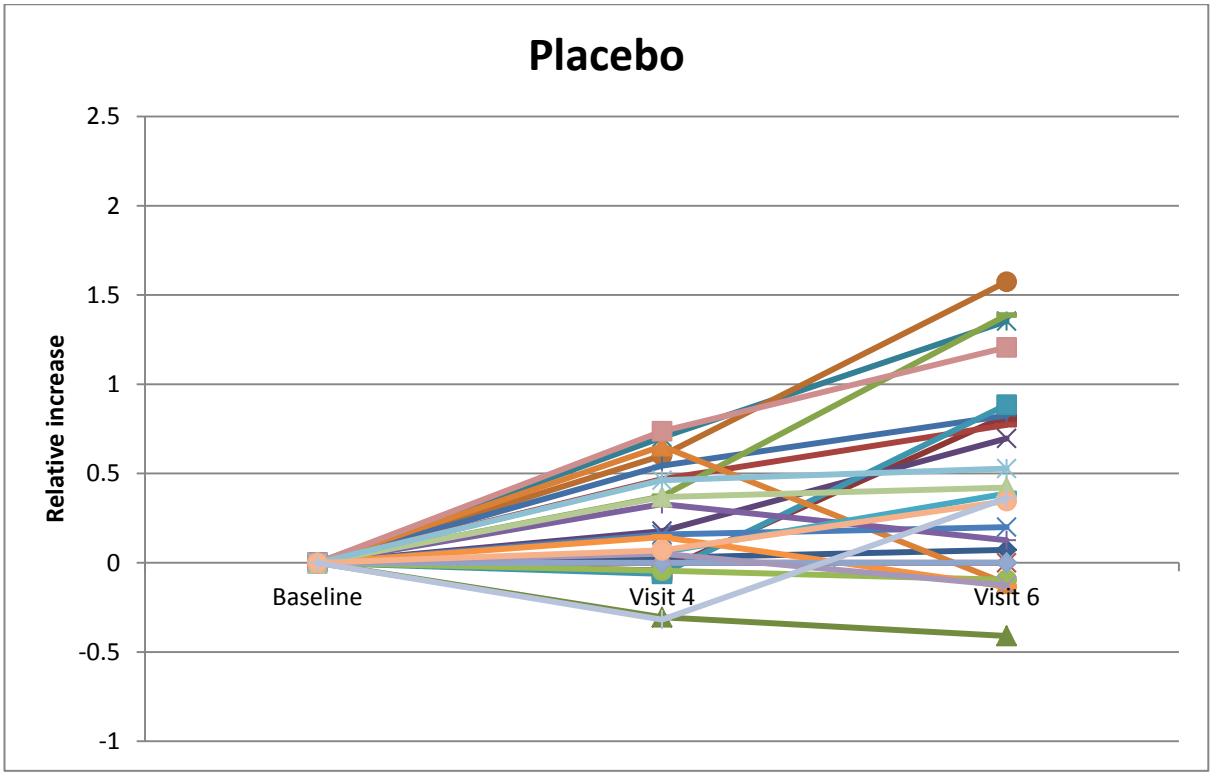

Figure 26 – Relative increase in COD (individual participants on placebo)

Table 15 below shows the peak walking distances for each arm of the study at the different stages. The baseline figure is the mean of Visit 1 and Visit 2 as outlined in the study protocol.

Time point	Allopurinol (95% CI)	Placebo (95% CI)	P
Baseline (metres)	315.5 (206.0 to 425.0)	362.6 (266.4 to 458.8)	0.24
Visit 4 (metres)	320.1 (209.0 to 431.2)	341.5 (256.9 to 426.1)	0.42
Visit 6 (metres)	347.4 (224.8 to 470.0)	358.4 (253.9 to 462.9)	0.61

Table 15 – PWD in metres at different study stages

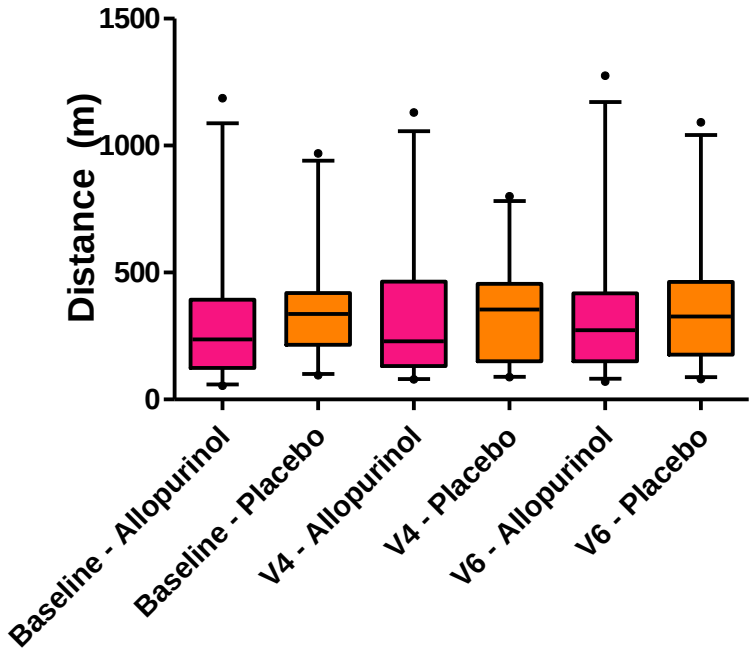

Figure 27 – PWD in metres at different study stages

The change in peak walking distance from baseline is shown in Table 16 and Figure 28.

	Allopurinol (95% CI)	**Placebo (95% CI)**	**P**
Mean change from Baseline to Visit 4 (metres)	-10.1 (-47.5 to 27.2)	19.3 (-19.4 to 58.1)	0.28
Mean change from Baseline to Visit 6 (metres)	17.1 (-18.5 to 52.8)	36.2 (-4.3 to 76.8)	0.34

Table 16 – Absolute change in PWD from baseline

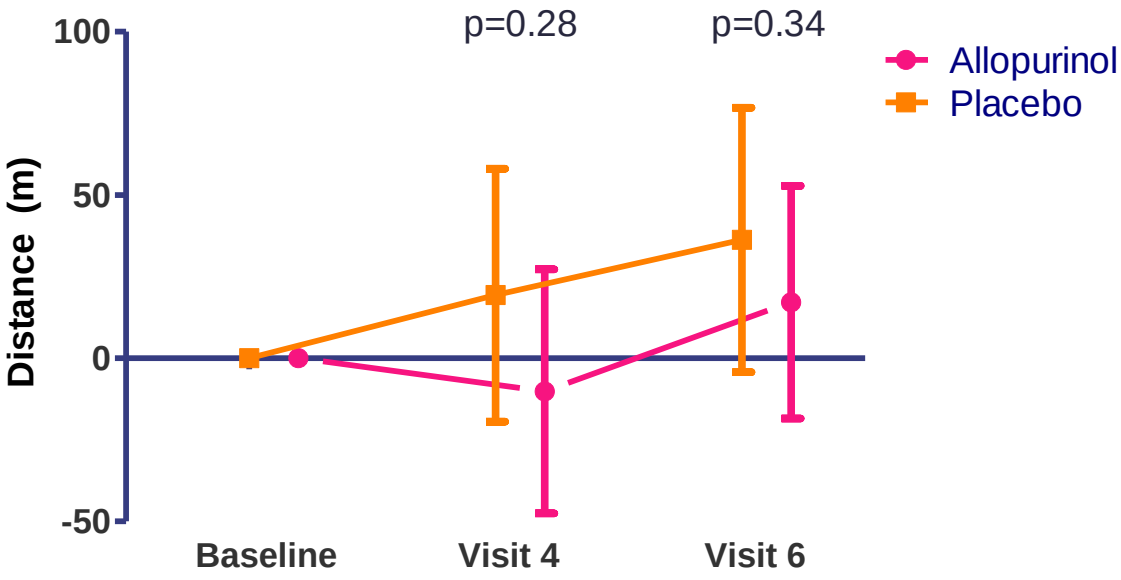

Figure 28 – Mean absolute change in PWD (vertical bars indicate 95% CI)

Given the variability in baseline walking distances noted in Table 12, the mean relative change was also calculated and is shown in Table 17 and Figure 29 below.

	Allopurinol (95% CI)	**Placebo (95% CI)**	**P**
Relative increase from Baseline to Visit 4	0.01 (-0.11 to 0.14)	0.12 (-0.04 to 0.27)	0.35
Relative increase from Baseline to Visit 6	0.14 (-0.07 to 0.34)	0.12 (0.01 to 0.24)	0.40

Table 17 – Relative increase in PWD from baseline

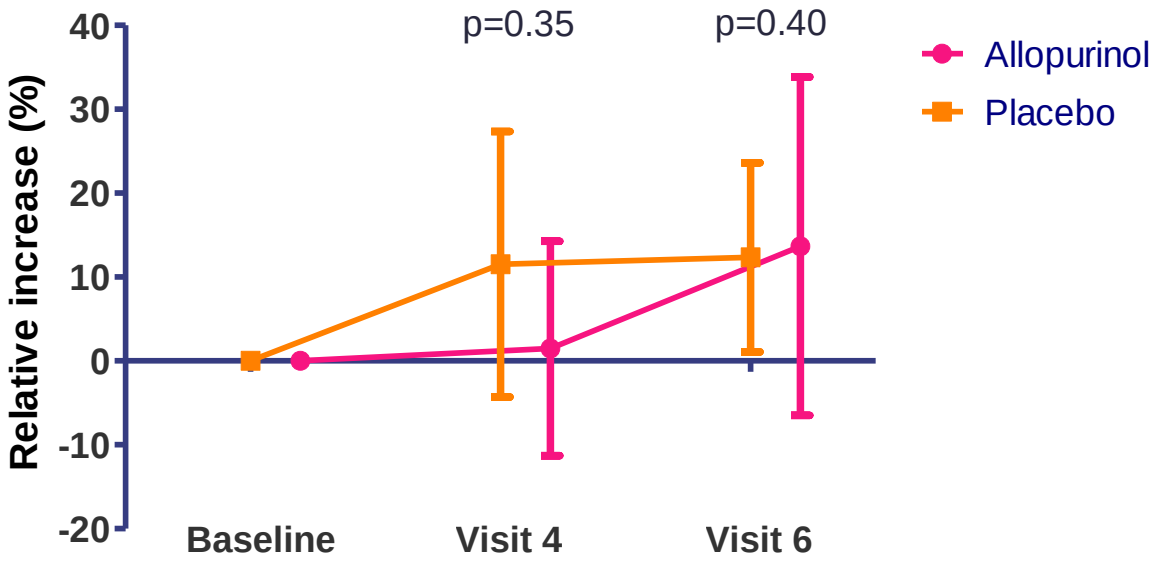

Figure 29 – Mean relative increase in PWD (vertical bars indicate 95% CI)

The individual change for each participant is shown in Figure 30 and Figure 31.

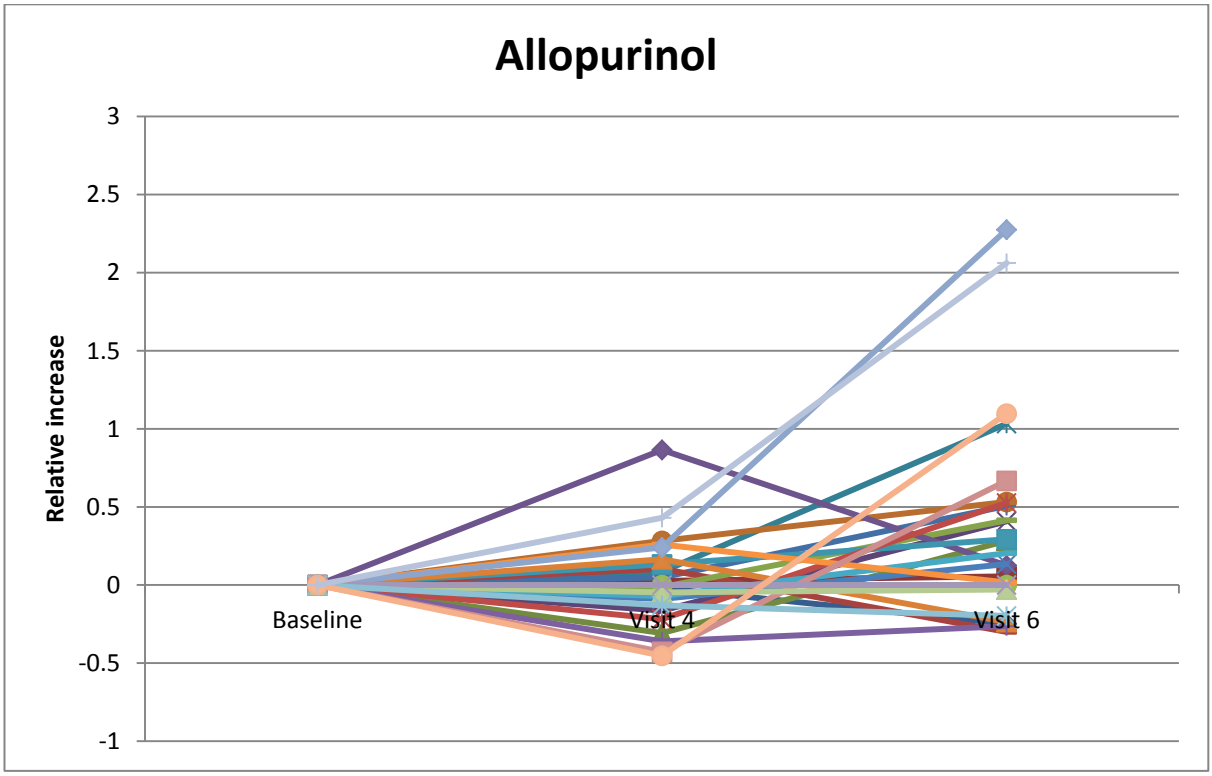

Figure 30 – Relative increase in PWD (individual participants on allopurinol)

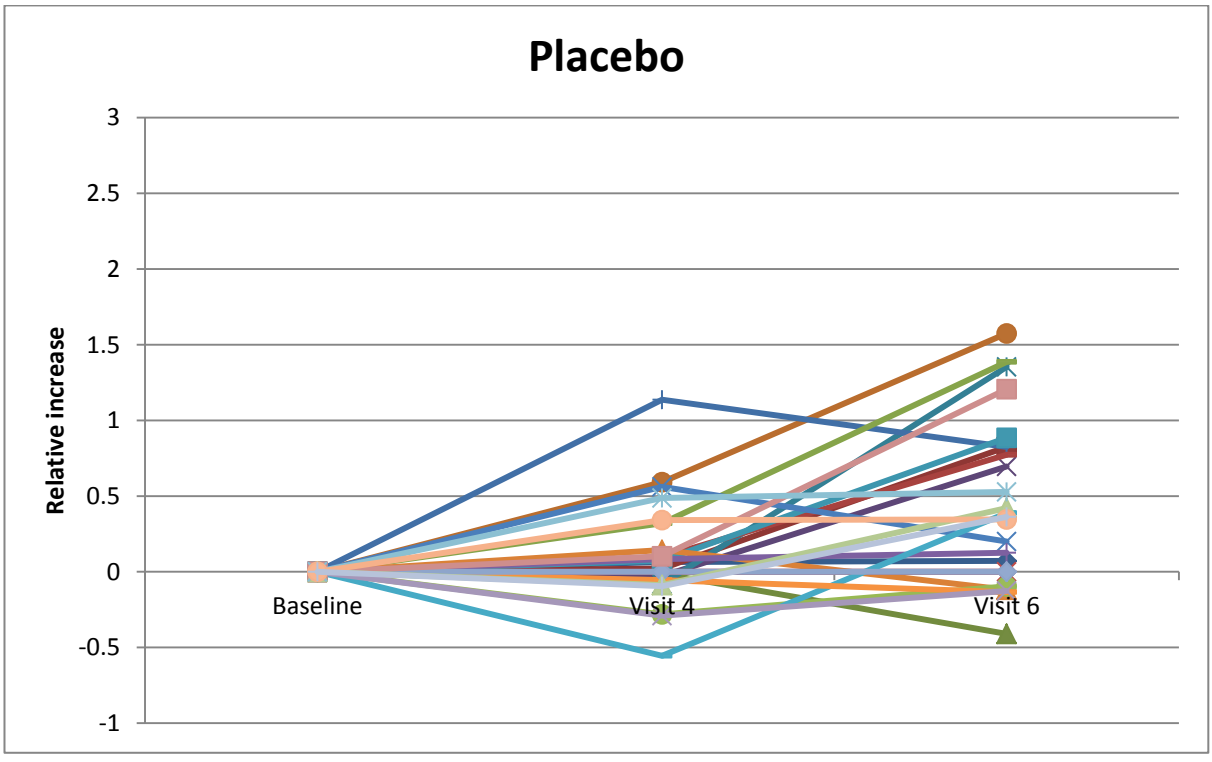

Figure 31 – Relative increase in PWD (individual participants on placebo)

To safeguard against the impact of any missing data, analysis of the primary outcome was also performed following multiple imputation. As demonstrated in Table 18 and Table 19, all P values remained non-significant following this analysis.

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p (imputed data)
Baseline (metres)	137.1 (82.0 to 192.2)	151.7 (105.4 to 198.0)	0.31
Visit 4 (metres)	155.7 (134.8 to 176.6)	174.4 (154.3 to 194.5)	0.39
Visit 6 (metres)	172.5 (145.4 to 199.7)	195.6 (175.3 to 215.9)	0.20

Table 18 – COD in metres at different study stages following multiple imputation

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p (imputed data)
Baseline (metres)	315.5 (206.0 to 425.0)	362.6 (266.4 to 458.8)	0.24
Visit 4 (metres)	307.7 (263.6 to 351.7)	373.7 (336.2 to 411.2)	0.13
Visit 6 (metres)	334.3 (285.7 to 383.0)	396.1 (351.5 to 440.8)	0.12

Table 19 – PWD in metres at different study stages following multiple imputation

3.5.2 Factor: above/below median baseline claudication onset

The median claudication onset distance at baseline was 98.4m. The participants were split into two groups – those below and those above this median level. The relative increases in both claudication onset distance and peak walking distance were then calculated.

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in COD from Baseline to Visit 4	Below median baseline COD	0.55 (0.25 to 0.85)	0.29 (0.07 to 0.51)	0.45
	Above median baseline COD	0.11 (-0.13 to 0.36)	0.18 (-0.02 to 0.38)	0.70
Relative increase in COD from Baseline to Visit 6	Below median baseline COD	0.53 (0.13 to 0.93)	0.74 (0.38 to 1.09)	0.35
	Above median baseline COD	0.27 (-0.22 to 0.76)	0.27 (-0.07 to 0.60)	0.75

Table 20 – Relative increase in COD split by low/high baseline COD

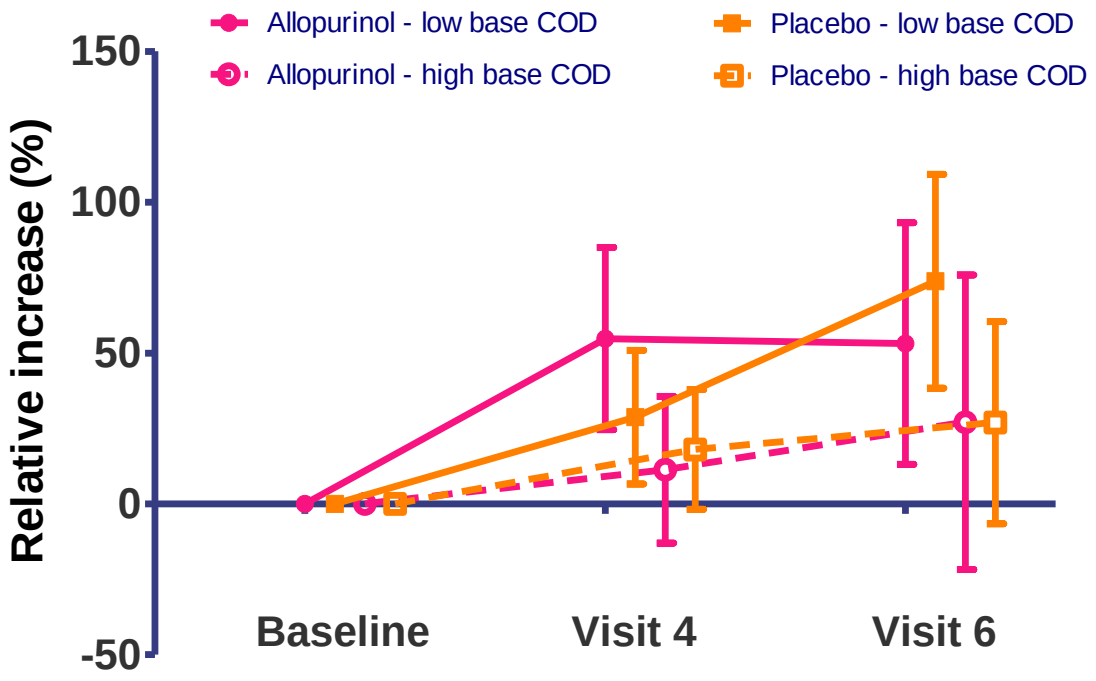

Figure 32 – Relative increase in COD split by low/high baseline COD

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in PWD from Baseline to Visit 4	Below median baseline COD	0.03 (-0.20 to 0.26)	0.29 (0.07 to 0.51)	0.45
	Above median baseline COD	0.00 (-0.14 to 0.14)	0.18 (-0.02 to 0.38)	0.75
Relative increase in PWD from Baseline to Visit 6	Below median baseline COD	0.24 (-0.14 to 0.63)	0.16 (-0.02 to 0.34)	0.74
	Above median baseline COD	0.02 (-0.12 to 0.17)	0.09 (-0.08 to 0.25)	0.48

Table 21 – Relative increase in PWD split by low/high baseline COD

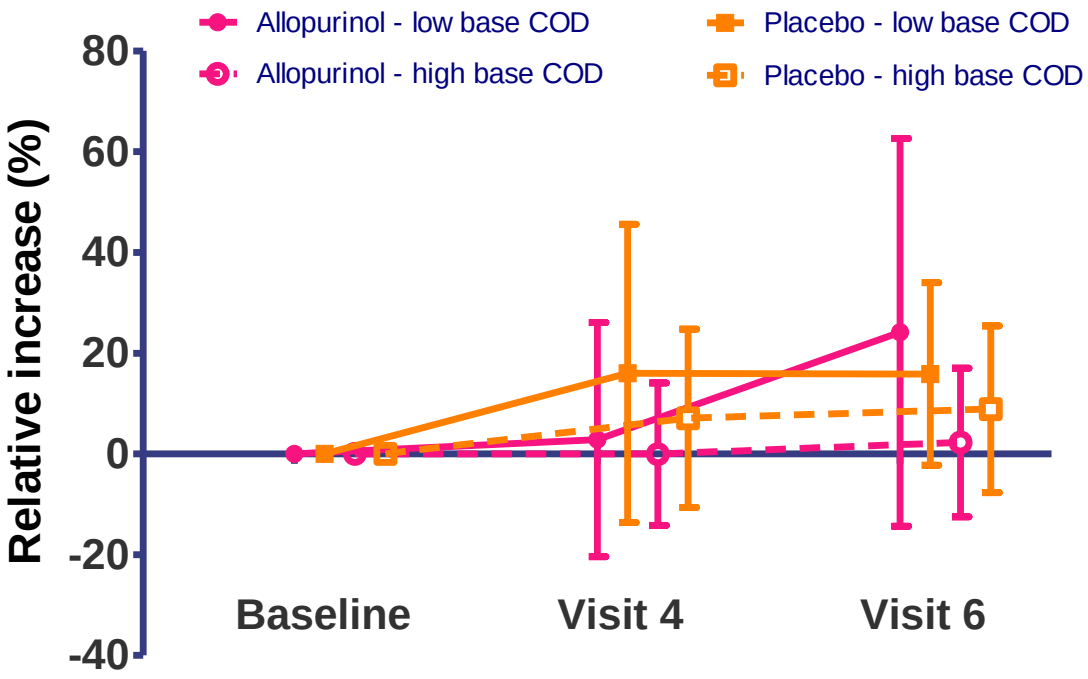

Figure 33 – Relative increase in PWD split by low/high baseline COD

3.5.3 Factor: above/below median baseline peak walking distance

The median peak walking distance at baseline was 278.1m. The participants were split into two groups – those below and those above this median level. The relative increases in both claudication onset distance and peak walking distance were then calculated.

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in COD from Baseline to Visit 4	Below median baseline PWD	0.49 (0.14 to 0.85)	0.20 (0.00 to 0.40)	0.41
	Above median baseline PWD	0.20 (-0.04 to 0.44)	0.28 (0.05 to 0.50)	0.82
Relative increase in COD from Baseline to Visit 6	Below median baseline PWD	0.37 (-0.10 to 0.84)	0.36 (0.03 to 0.69)	0.79
	Above median baseline PWD	0.44 (0.01 to 0.87)	0.70 (0.27 to 1.09)	0.23

Table 22 – Relative increase in COD split by low/high baseline PWD

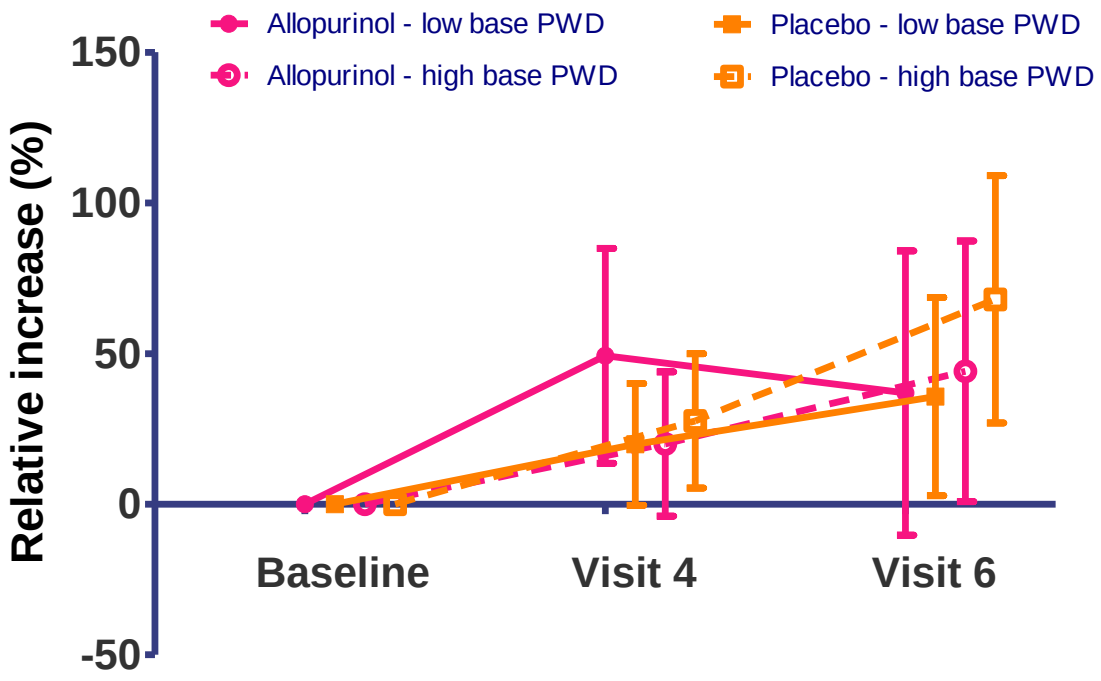

Figure 34 – Relative increase in COD split by low/high baseline PWD

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in PWD from Baseline to Visit 4	Below median baseline PWD	0.08 (-0.17 to 0.32)	0.15 (-0.14 to 0.44)	0.88
	Above median baseline PWD	-0.04 (-0.18 to 0.10)	0.07 (-0.08 to 0.23)	0.28
Relative increase in PWD from Baseline to Visit 6	Below median baseline PWD	0.24 (-0.19 to 0.67)	0.10 (-0.08 to 0.28)	0.93
	Above median baseline PWD	0.04 (-0.09 to 0.17)	0.16 (0.00 to 0.32)	0.23

Table 23 – Relative increase in PWD split by low/high baseline PWD

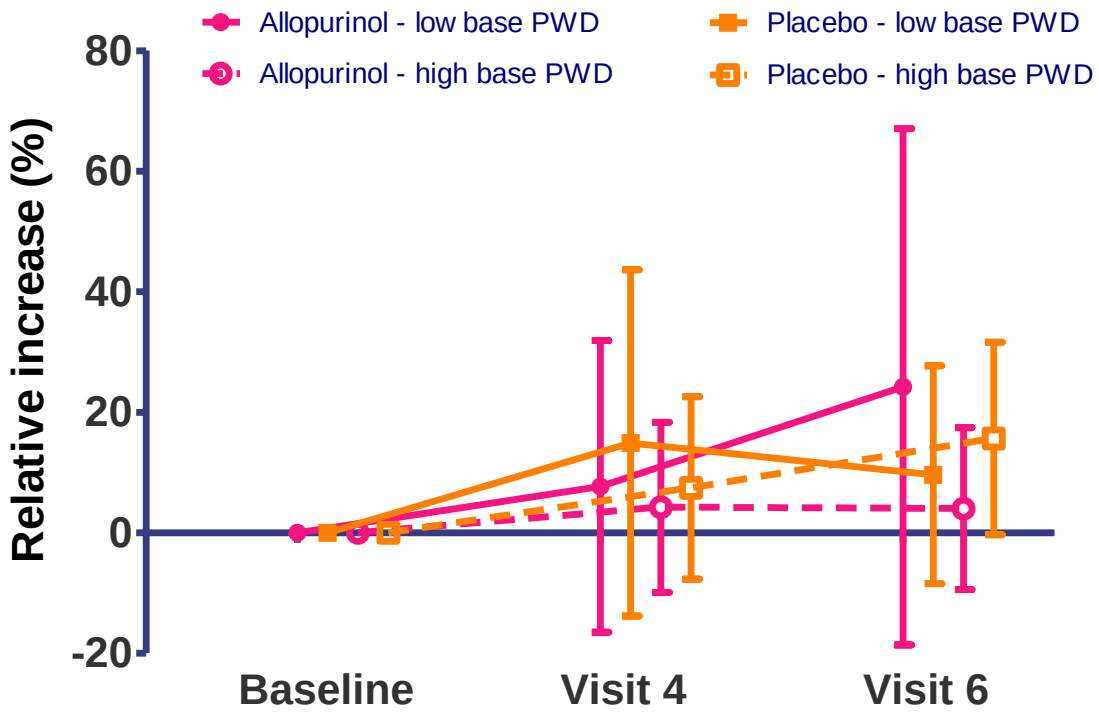

Figure 35 – Relative increase in PWD split by low/high baseline PWD

3.5.4 Factor: above/below median baseline uric acid

The median uric acid at baseline was 0.36mmol/l. The participants were split into two groups – those below and those above this median level. The relative increases in both claudication onset distance and peak walking distance were then calculated.

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in COD from Baseline to Visit 4	Below median baseline urate	0.37 (0.12 to 0.61)	0.17 (-0.05 to 0.38)	0.32
	Above median baseline urate	0.30 (-0.10 to 0.71)	0.28 (0.08 to 0.48)	0.78
Relative increase in COD from Baseline to Visit 6	Below median baseline urate	0.33 (0.06 to 0.60)	0.27 (-0.09 to 0.62)	0.85
	Above median baseline urate	0.51 (-0.15 to 1.16)	0.67 (0.32 to 1.01)	0.31

Table 24 – Relative increase in COD split by low/high baseline urate

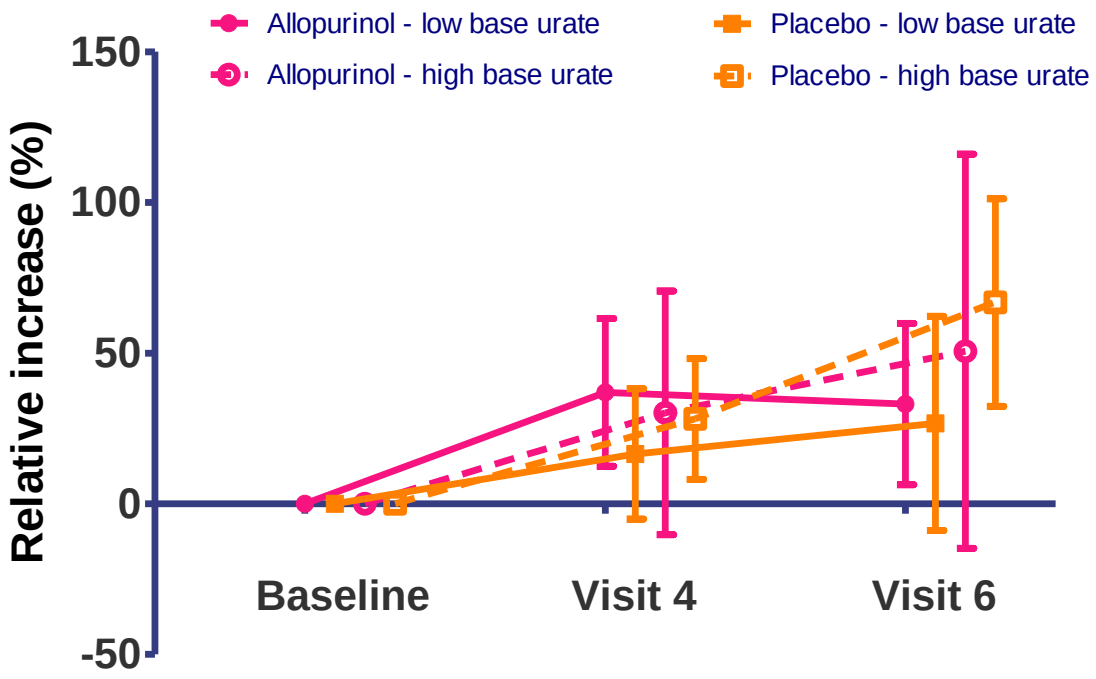

Figure 36 – Relative increase in COD split by low/high baseline urate

		Allopurinol (95% CI)	Placebo (95% CI)	P
Relative increase in PWD from Baseline to Visit 4	Below median baseline urate	-0.13 (-0.25 to -0.01)	0.11 (-0.20 to 0.42)	0.11
	Above median baseline urate	0.21 (0.00 to 0.42)	0.12 (-0.08 to 0.33)	0.69
Relative increase in PWD from Baseline to Visit 6	Below median baseline urate	-0.02 (-0.14 to 0.09)	0.08 (-0.16 to 0.33)	0.39
	Above median baseline urate	0.35 (-0.11 to 0.80)	0.15 (-0.02 to 0.28)	0.88

Table 25 – Relative increase in PWD split by low/high baseline urate

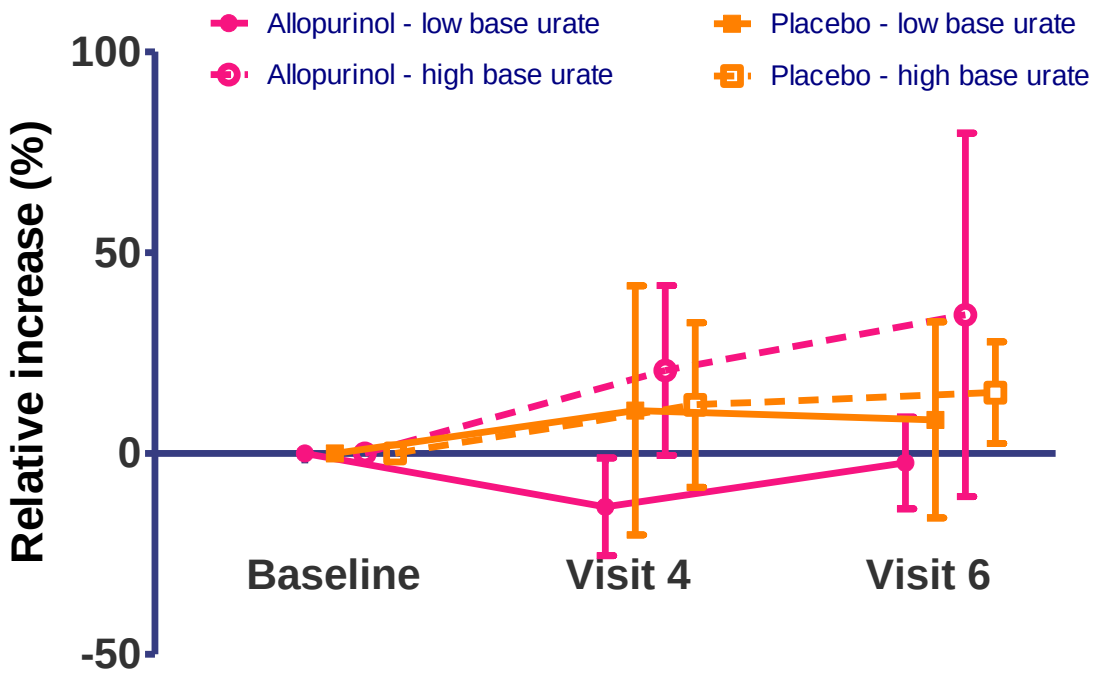

Figure 37 – Relative increase in PWD split by low/high baseline urate

When considering Figure 37 above, it can be seen that the lines for PWD in the allopurinol group appear to be slightly diverging, albeit with overlapping confidence intervals. To check for any difference between the low and high baseline urate sub-populations the baseline characteristics for these two groups were compared – no significant difference in any factor was found.

Also considered for those in the active treatment group was whether the percentage reduction in uric acid (i.e. the relative response to allopurinol) made any difference to the change in either claudication onset or peak walking distance. The median reduction in uric acid for those on allopurinol was 57.0% and this was used to split the active group in two. As can been seen in Figure 38 and Figure 39 below there was no significant difference between those that had a reduction in uric acid either above or below this median level.

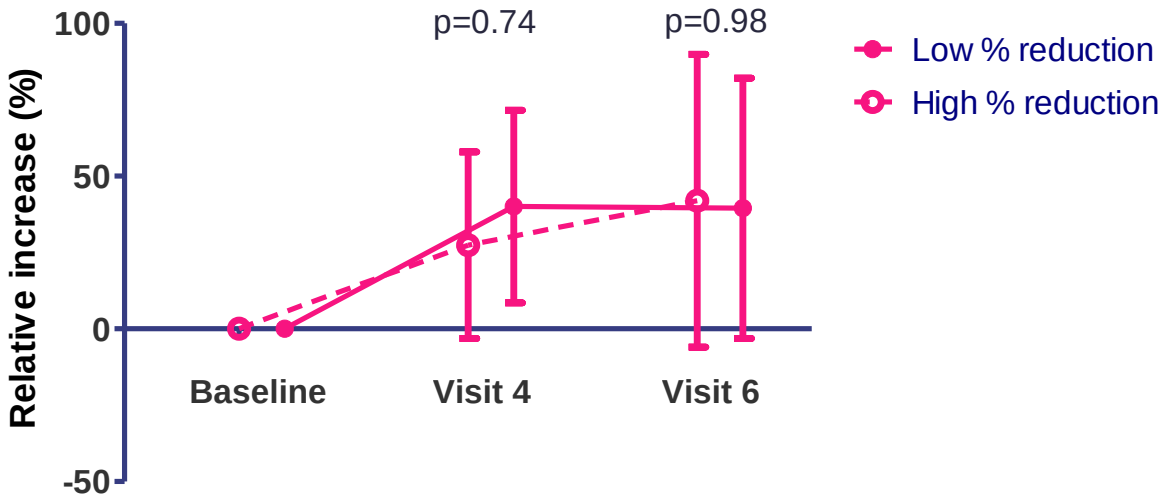

Figure 38 – Relative increase in COD for low vs high reduction in uric acid

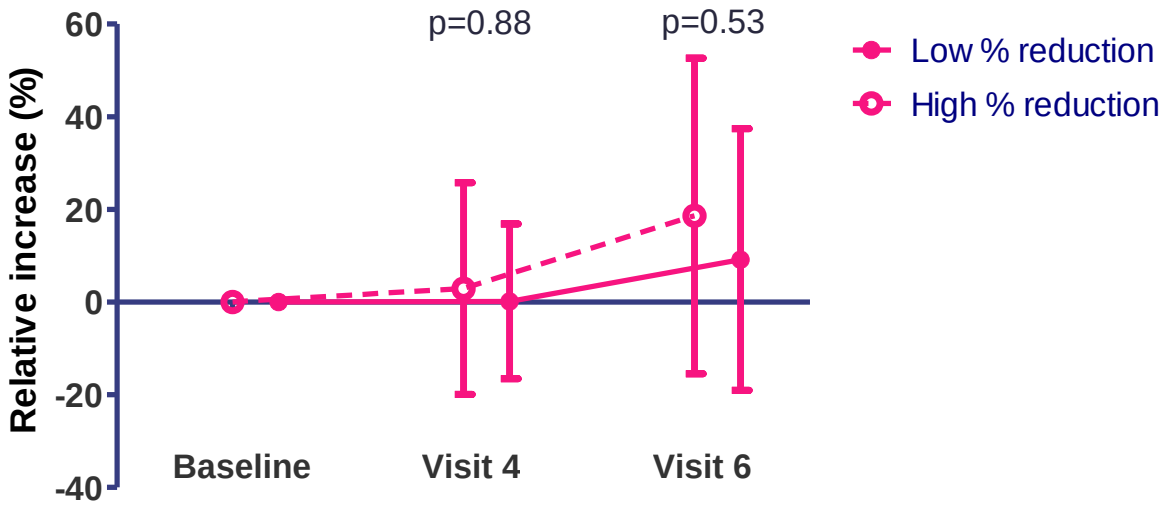

Figure 39 – Relative increase in PWD for low vs high reduction in uric acid

3.5.5 Factor: above/below median baseline systolic BP

The median systolic BP at baseline was 154.5mmHg. The participants were split into two groups – those below and those above this median level. The relative increases in both claudication onset distance and peak walking distance were then calculated.

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in COD from Baseline to Visit 4	Below median baseline systolic BP	0.32 (0.02 to 0.62)	0.28 (0.09 to 0.46)	0.95
	Above median baseline systolic BP	0.36 (0.04 to 0.68)	0.19 (-0.04 to 0.42)	0.70
Relative increase in COD from Baseline to Visit 6	Below median baseline systolic BP	0.36 (-0.10 to 0.82)	0.75 (0.39 to 1.12)	0.05
	Above median baseline systolic BP	0.45 (0.01 to 0.89)	0.25 (-0.05 to 0.56)	0.61

Table 26 – Relative increase in COD split by low/high baseline systolic BP

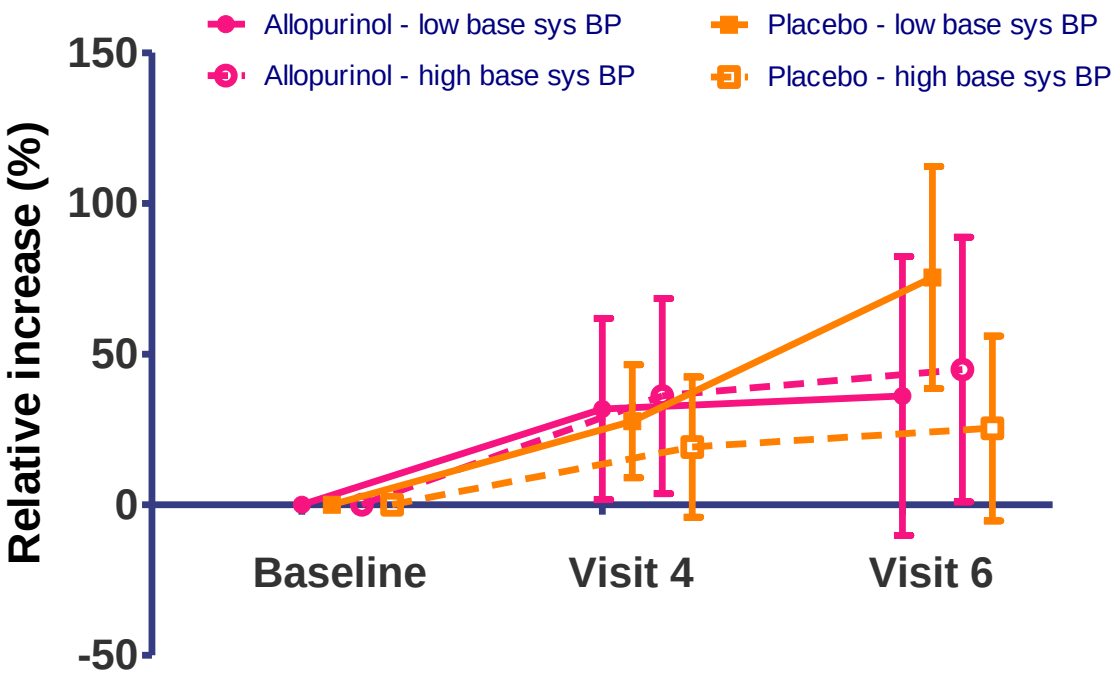

Figure 40 – Relative increase in COD split by low/high baseline systolic BP

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in PWD from Baseline to Visit 4	Below median baseline systolic BP	-0.01 (-0.12 to 0.10)	0.13 (-0.17 to 0.43)	0.52
	Above median baseline systolic BP	0.03 (-0.21 to 0.28)	0.10 (-0.07 to 0.28)	0.57
Relative increase in PWD from Baseline to Visit 6	Below median baseline systolic BP	0.04 (-0.08 to 0.16)	0.15 (-0.03 to 0.33)	0.44
	Above median baseline systolic BP	0.23 (-0.17 to 0.62)	0.10 (-0.07 to 0.27)	0.88

Table 27 – Relative increase in PWD split by low/high baseline systolic BP

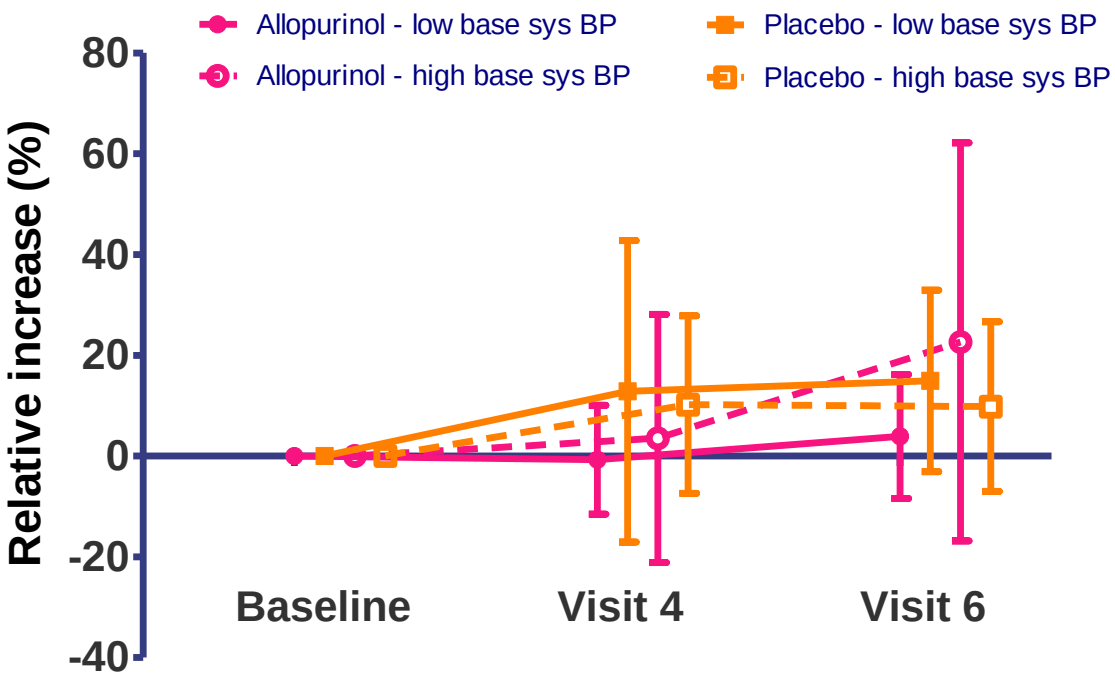

Figure 41 – Relative increase in PWD split by low/high baseline systolic BP

3.5.6 Factor: above/below median baseline ABI

The median ABI at baseline was 0.60. The participants were split into two groups – those below and those above this median level. The relative increases in both claudication onset distance and peak walking distance were then calculated.

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in COD from Baseline to Visit 4	Below median baseline ABI	0.29 (-0.02 to 0.60)	0.17 (-0.07 to 0.41)	1.00
	Above median baseline ABI	0.40 (0.08 to 0.71)	0.29 (0.10 to 0.47)	0.61
Relative increase in COD from Baseline to Visit 6	Below median baseline ABI	0.51 (-0.03 to 1.04)	0.29 (0.02 to 0.55)	0.87
	Above median baseline ABI	0.30 (0.00 to 0.60)	0.69 (0.29 to 1.08)	0.13

Table 28 – Relative increase in COD split by low/high baseline ABI

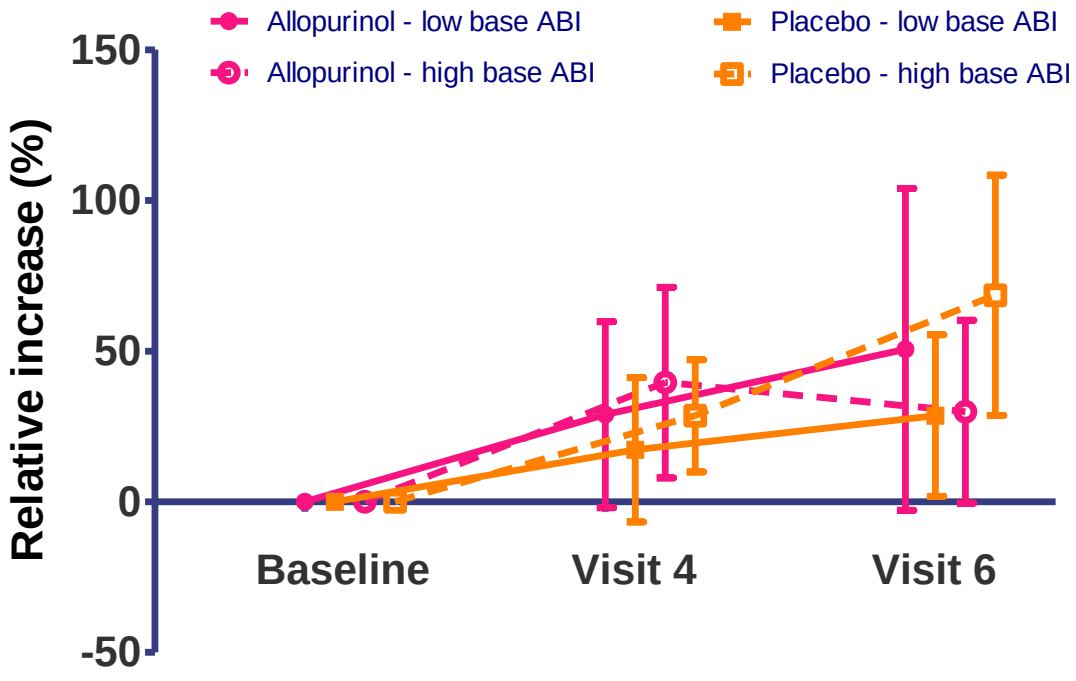

Figure 42 – Relative increase in COD split by low/high baseline ABI

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in PWD from Baseline to Visit 4	Below median baseline ABI	0.05 (-0.12 to 0.22)	0.16 (-0.16 to 0.48)	0.87
	Above median baseline ABI	-0.02 (-0.25 to 0.20)	0.08 (-0.09 to 0.25)	0.26
Relative increase in PWD from Baseline to Visit 6	Below median baseline ABI	0.15 (-0.14 to 0.43)	0.14 (-0.07 to 0.34)	0.77
	Above median baseline ABI	0.13 (-0.21 to 0.47)	0.11 (-0.04 to 0.27)	0.35

Table 29 – Relative increase in PWD split by low/high baseline ABI

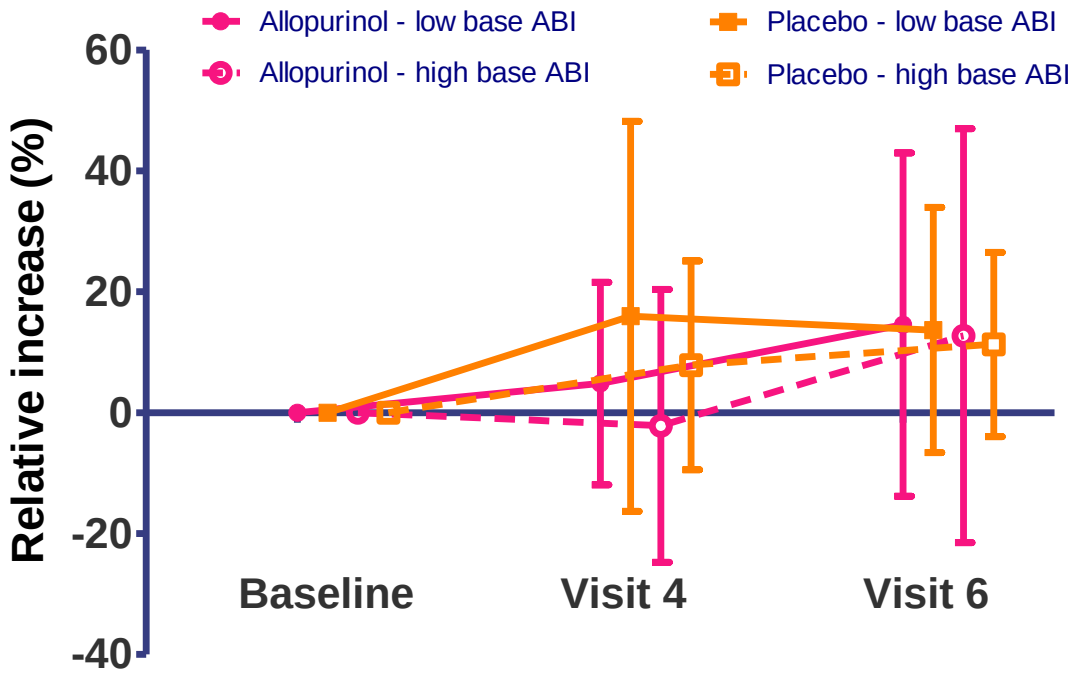

Figure 43 – Relative increase in PWD split by low/high baseline ABI

3.6 Six Minute Walk Test

Data from OpenClinica provided a total walking distance in metres for each 6MWT. The absolute and relative change in six-minute walk distance (6MWD) was then calculated, comparing those on allopurinol with those on placebo.

The data was initially analysed without any subdivision (section 3.6.1). It was then sub-divided by two different factors:

- Factor: above/below median baseline six-minute walk distance (section 3.6.2)
- Factor: above/below median baseline uric acid (section 3.6.3)

3.6.1 Without any sub-division by factor

Table 30 and Figure 44 below show the six minute walk test distance reached for each treatment arm at different stages of the study.

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline (metres)	401.2 (371.7 to 430.7)	389.1 (369.2 to 409.1)	0.72
Visit 4 (metres)	396.3 (366.1 to 426.6)	395.6 (376.3 to 414.8)	0.97
Visit 6 (metres)	398.2 (374.4 to 422.1)	399.9 (375.8 to 424.0)	0.92

Table 30 – 6MWD in metres at different study stages

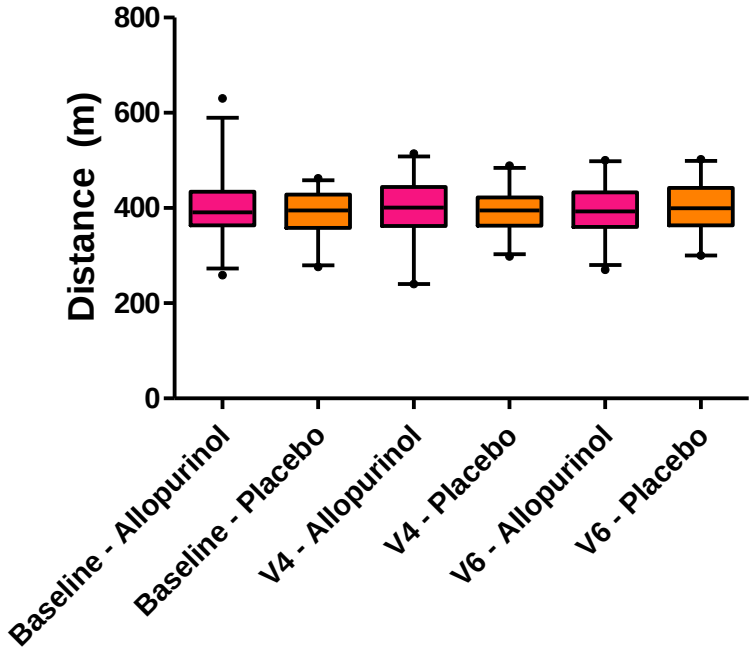

Figure 44 – 6MWD in metres at different study stages

The change in six minute walk test distance from baseline is shown in Table 31 and Figure 45.

	Allopurinol (95% CI)	**Placebo (95% CI)**	**p**
Mean change from Baseline to Visit 4 (metres)	4.4 (-10.5 to 19.4)	8.7 (-2.4 to 19.8)	0.73
Mean change from Baseline to Visit 6 (metres)	6.4 (-3.9 to 16.6)	13.1 (1.0 to 25.1)	0.63

Table 31 – Absolute change in 6MWD from baseline

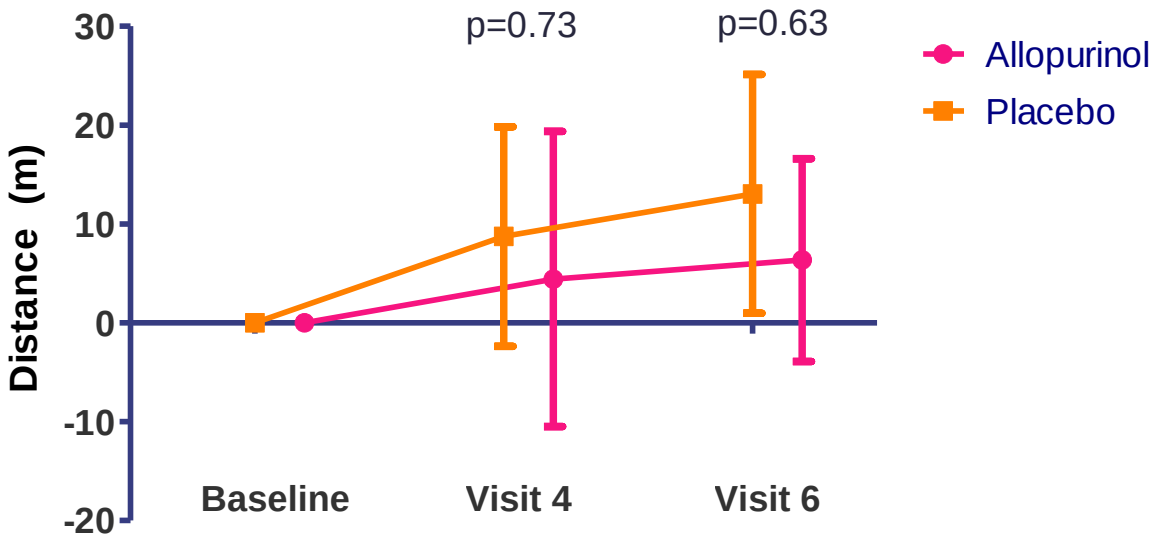

Figure 45 – Mean absolute change in 6MWD (vertical bars indicate 95% CI)

Given the range of baseline walking distances noted in Table 30, the mean relative change was calculated and is shown in Table 32 and Figure 46 below.

	Allopurinol (95% CI)	**Placebo (95% CI)**	**p**
Relative increase from Baseline to Visit 4	0.01 (-0.03 to 0.05)	0.03 (0.00 to 0.06)	0.70
Relative increase from Baseline to Visit 6	0.02 (-0.01 to 0.05)	0.03 (0.00 to 0.07)	0.56

Table 32 – Relative increase in 6MWD from baseline

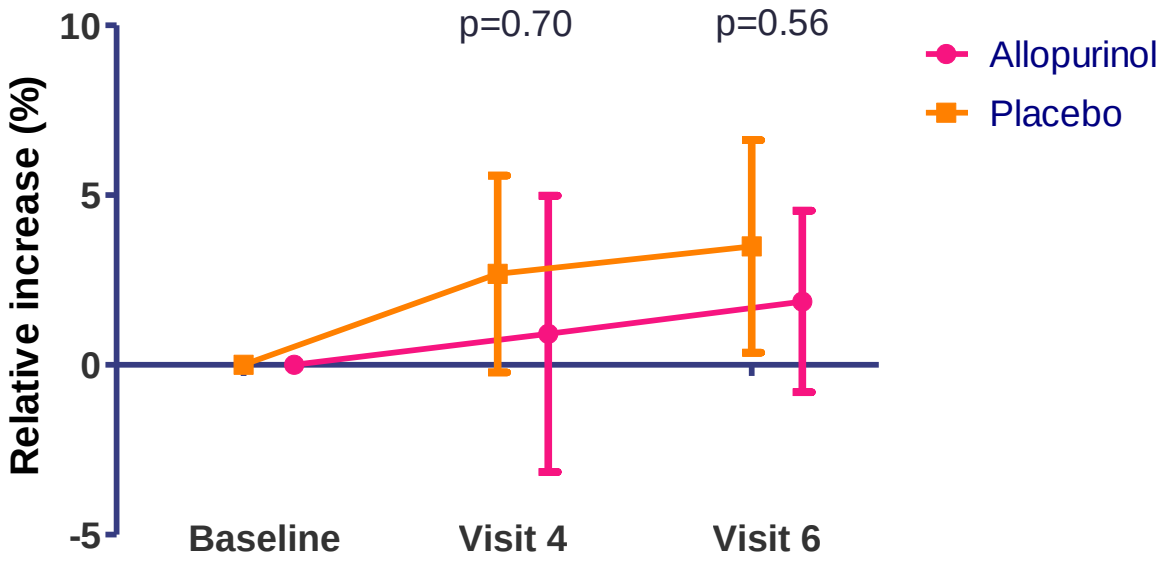

Figure 46 – Relative increase in 6MWD (vertical bars indicate 95% CI)

The individual change for each participant is shown in Figure 47 and Figure 48 below.

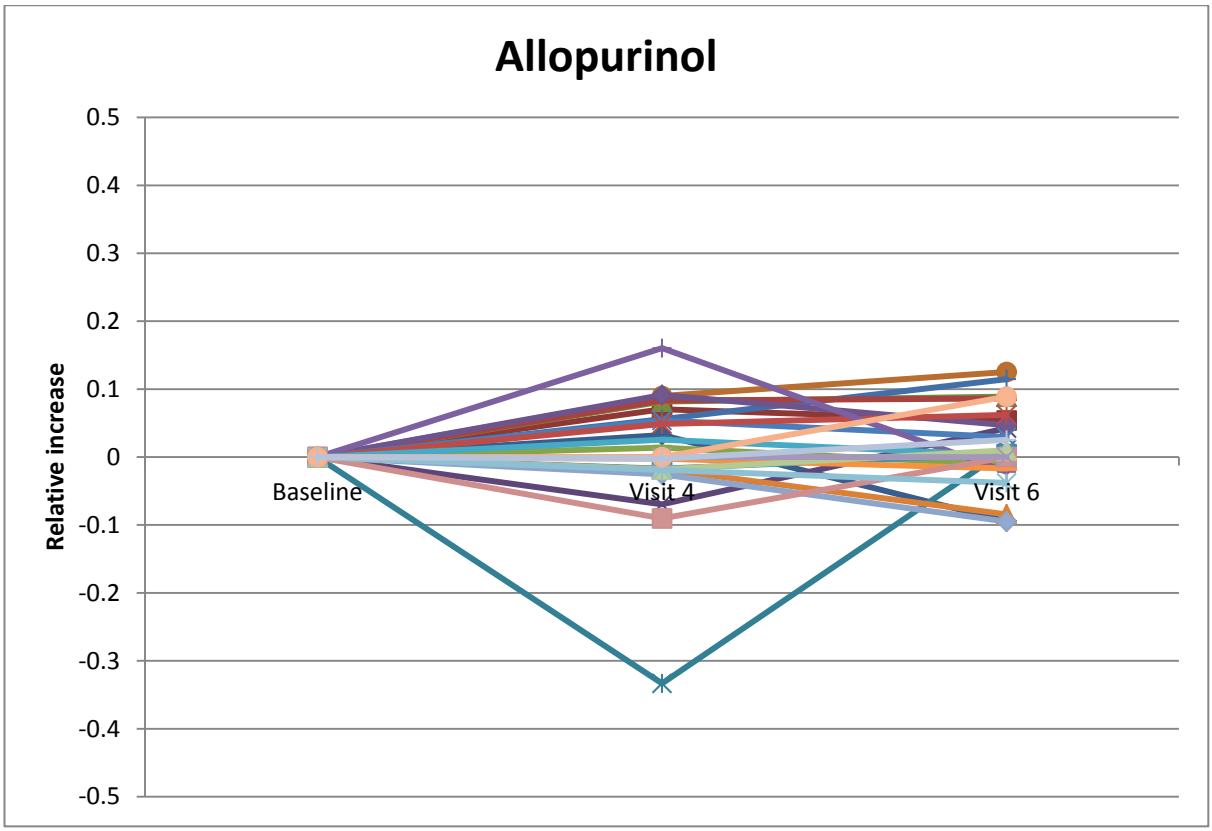

Figure 47 – Relative increase in 6MWD (individual participants on allopurinol)

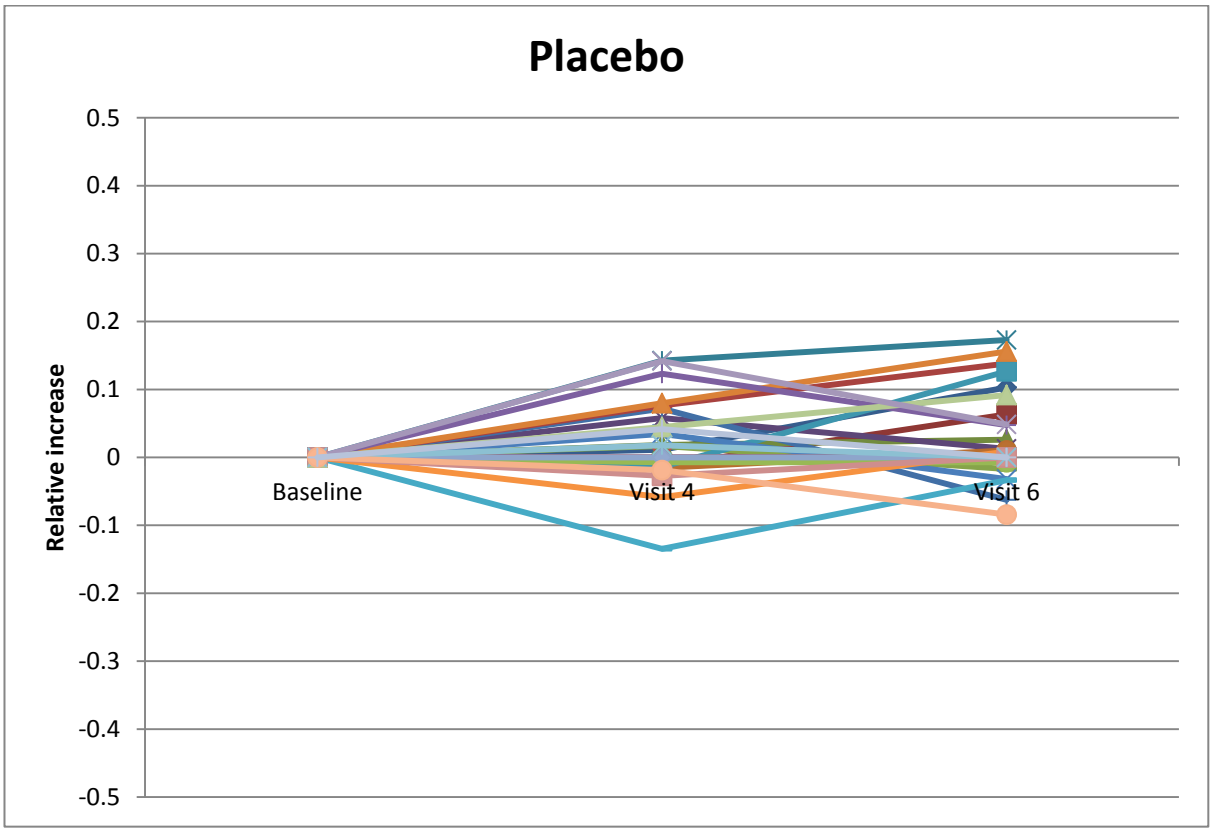

Figure 48 – Relative increase in 6MWD (individual participants on placebo)

3.6.2 Factor: above/below median baseline six-minute walk distance

The median baseline six minute walk distance was 393m. The participants were split into two groups – those below and those above this median level. The relative increases in walking distance for each group were then calculated.

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in 6MWD from Baseline to Visit 4	Below median baseline 6MWD	-0.01 (-0.09 to 0.07)	0.06 (0.02 to 0.11)	0.12
	Above median baseline 6MWD	0.03 (0.00 to 0.06)	0.01 (-0.03 to 0.04)	0.73
Relative increase in 6MWD from Baseline to Visit 6	Below median baseline 6MWD	0.01 (-0.03 to 0.06)	0.01 (-0.06 to 0.07)	0.40
	Above median baseline 6MWD	0.02 (-0.01 to 0.06)	0.05 (0.01 to 0.09)	0.43

Table 33 – Relative increase in 6MWD split by low/high baseline 6MWD

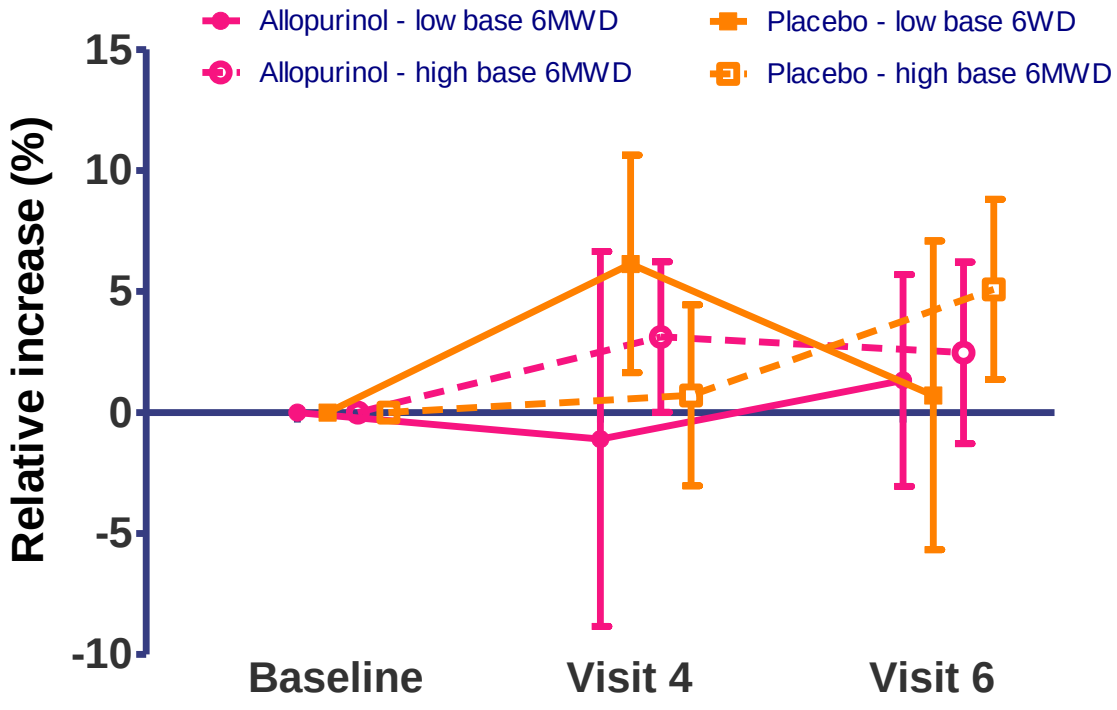

Figure 49 – Relative increase in 6MWD split by low/high baseline 6MWD

3.6.3 Factor: above/below median baseline uric acid

The median uric acid at baseline was 0.36mmol/l. The participants were split into two groups – those below and those above this median level. The relative increases in walking distance for each group were then calculated.

		Allopurinol (95% CI)	Placebo (95% CI)	p
Relative increase in 6MWD from Baseline to Visit 4	Below median baseline urate	0.00 (-0.07 to 0.08)	0.03 (-0.01 to 0.07)	0.85
	Above median baseline urate	0.02 (-0.02 to 0.06)	0.02 (-0.02 to 0.07)	0.74
Relative increase in 6MWD from Baseline to Visit 6	Below median baseline urate	0.02 (-0.01 to 0.06)	0.05 (0.00 to 0.09)	0.36
	Above median baseline urate	0.01 (-0.03 to 0.06)	0.03 (-0.02 to 0.07)	1.00

Table 34 – Relative increase in 6MWD split by low/high baseline urate

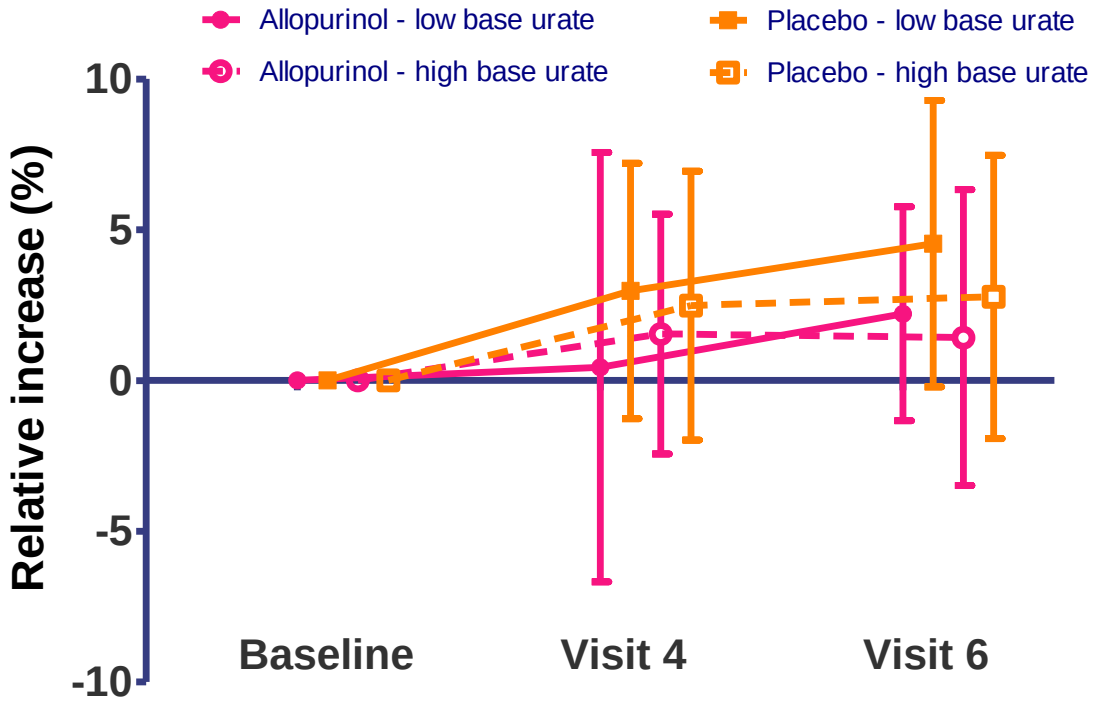

Figure 50 – Relative increase in 6MWD split by low/high baseline urate

Also considered for the active treatment group was whether the percentage reduction in uric acid (i.e. the relative response to allopurinol) made any difference to the change in six minute walk distance. The median reduction in uric acid for those on allopurinol was 57.0% and this was used to split the active group in two. As can been seen in Figure 51 below there was no significant difference between those that had a reduction in uric acid either above or below this median level.

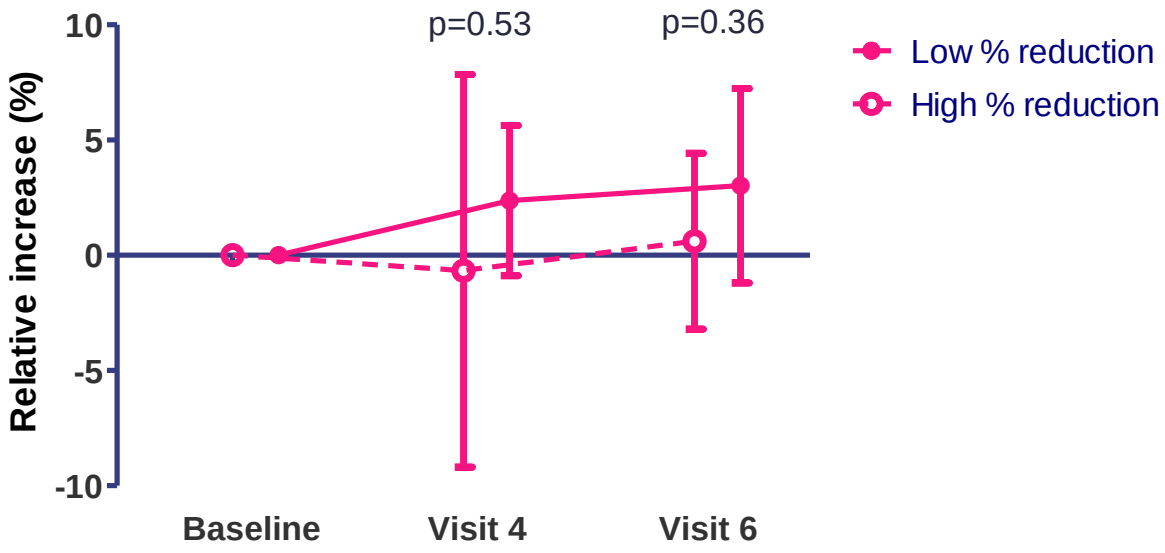

Figure 51 – Relative increase in 6MWD for low vs high reduction in uric acid

3.7 Blood pressure

As outlined in Table 7, there was no significant difference in blood pressure readings at baseline in the two treatment groups. Blood pressure had not been identified as a key outcome measure in the study protocol therefore it was not re-measured in the same way as many other characteristics at the end of the study. A blood pressure was however checked prior to six-minute walk testing at the start and end of the study. It should be noted though that for the vast majority of patients this followed FMD testing and therefore was a measurement taken after the use of GTN in the latter part of that test.

Within that limitation, comparison of these post-GTN blood pressures is shown in Table 35 (comparison between allopurinol and placebo at start and end of study), Table 36 (comparison between start and end of study by treatment group) and illustrated in Figure 52 below.

	Allopurinol Visit 2 (95% CI)	**Placebo Visit 2 (95% CI)**	**p**	**Allopurinol Visit 6 (95% CI)**	**Placebo Visit 6 (95% CI)**	**p**
Systolic BP (mmHg)	118 (110 to 126)	121 (113 to 130)	0.54	123 (116 to 131)	122 (116 to 127)	0.71
Diastolic BP (mmHg)	67 (63 to 72)	70 (66 to 74)	0.30	68 (64 to 72)	71 (68 to 75)	0.26

Table 35 – Post-GTN pre-6MWT blood pressures (allopurinol vs placebo)

	Allopurinol Visit 2 (95% CI)	Allopurinol Visit 6 (95% CI)	p	Placebo Visit 2 (95% CI)	Placebo Visit 6 (95% CI)	p
Systolic BP (mmHg)	118 (110 to 126)	123 (116 to 131)	0.30	121 (113 to 130)	122 (116 to 127)	0.93
Diastolic BP (mmHg)	67 (63 to 72)	68 (64 to 72)	0.81	70 (66 to 74)	71 (68 to 75)	0.72

Table 36 – Post-GTN pre-6MWT blood pressures (start vs end of study)

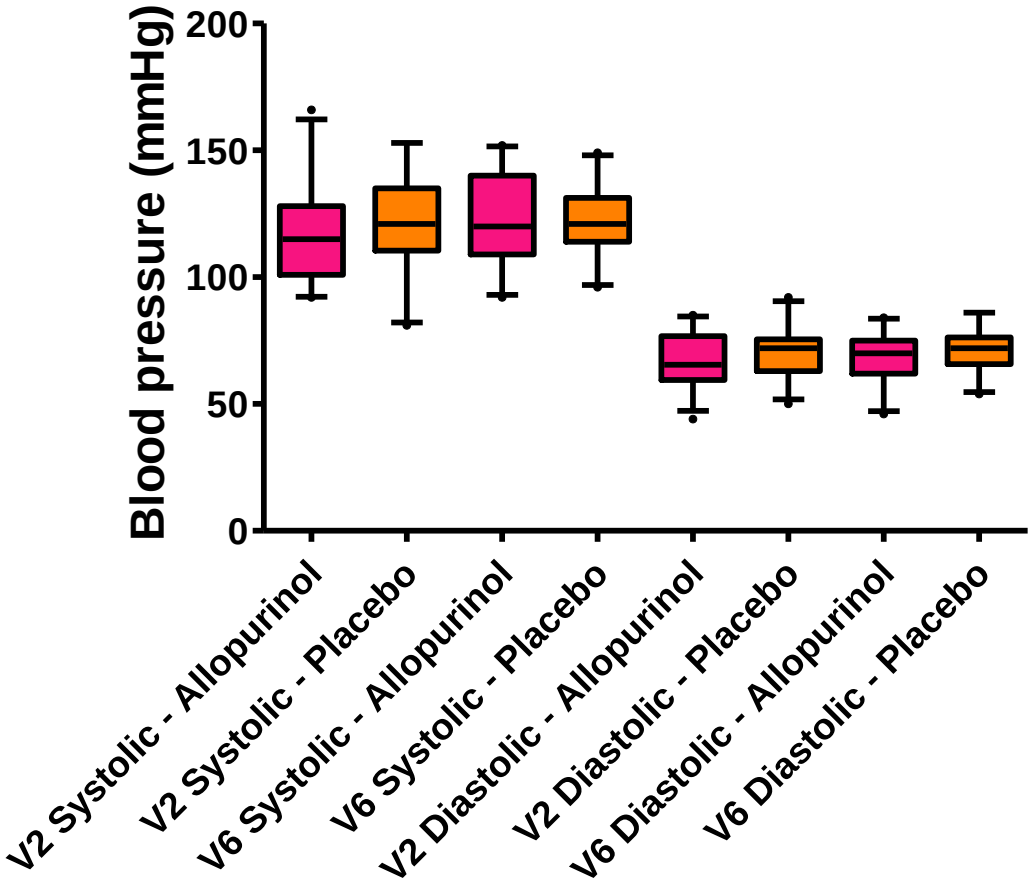

Figure 52 – Post-GTN pre-6MWT blood pressures

3.8 FMD

Analysis was carried out as described in section 2.8.6, for both post-cuff deflation (Table 37 and Figure 53) and post-GTN (Table 38 and Figure 54) flow-mediated dilatation.

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline vessel size (mm)	4.66 (4.34 to 4.98)	4.24 (3.75 to 4.73)	0.14
Absolute change post-cuff at baseline (mm)	0.38 (0.25 to 0.51)	0.34 (0.19 to 0.50)	0.65
Relative change post-cuff at baseline (%)	8.33 (5.87 to 10.79)	9.61 (4.92 to 14.30)	0.84
Absolute change post-cuff at Visit 5 (mm)	0.24 (0.16 to 0.31)	0.24 (0.13 to 0.34)	0.66
Relative change post-cuff at Visit 5 (%)	4.85 (3.46 to 6.24)	5.61 (3.54 to 7.69)	0.32
Relative change **compared to baseline** post-cuff at Visit 5 (%)	-0.29 (-0.53 to -0.06)	-0.15 (-0.59 to 0.28)	0.42
Absolute change post-cuff at Visit 6 (mm)	0.41 (0.30 to 0.52)	0.25 (0.14 to 0.36)	0.06
Relative change post-cuff at Visit 6 (%)	8.92 (6.43 to 11.40)	6.55 (3.49 to 9.61)	0.27
Relative change **compared to baseline** post-cuff at Visit 6 (%)	0.41 (-0.30 to 1.11)	-0.28 (-0.64 to 0.09)	0.32

Table 37 – Baseline vessel diameter and percentage change throughout study (cuff)

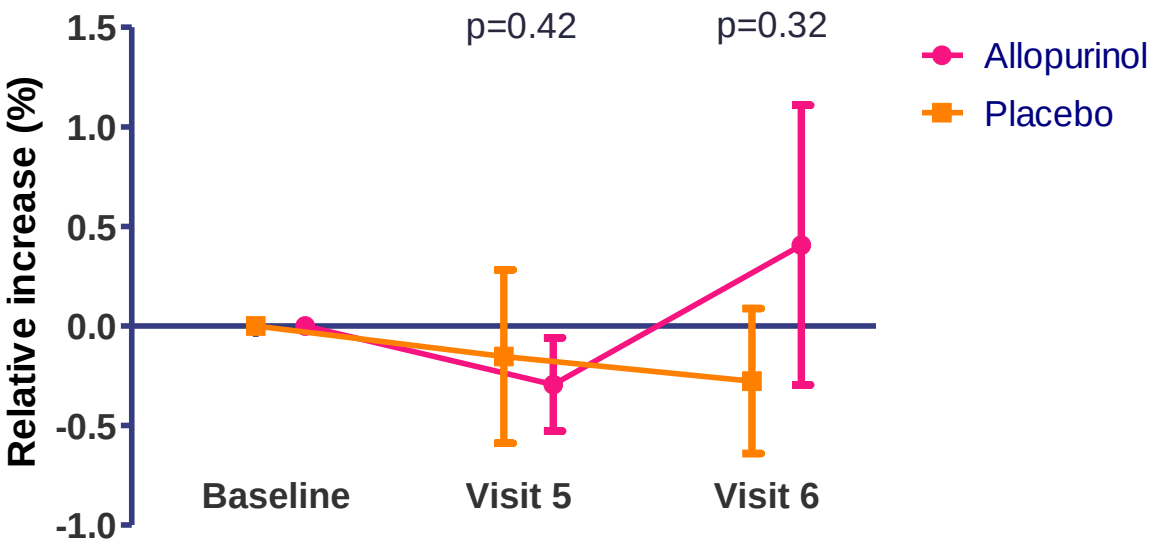

Figure 53 – Relative change in vessel diameter throughout study (cuff)

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline vessel size (mm)	4.80 (4.49 to 5.11)	4.45 (3.96 to 4.94)	0.13
Absolute change post-GTN at baseline (mm)	0.46 (0.39 to 0.53)	0.45 (0.36 to 0.55)	0.35
Relative change post-GTN at baseline (%)	9.94 (7.92 to 11.96)	11.04 (7.48 to 14.60)	0.96
Absolute change post-GTN at Visit 5 (mm)	0.33 (0.26 to 0.40)	0.45 (0.35 to 0.55)	0.52
Relative change post-GTN at Visit 5 (%)	6.57 (5.10 to 8.05)	10.50 (7.73 to 13.27)	**0.01**
Relative change **compared to baseline** post-GTN at Visit 5 (%)	-0.25 (-0.47 to -0.02)	0.02 (-0.39 to 0.43)	0.11
Absolute change post-GTN at Visit 6 (mm)	0.41 (0.32 to 0.50)	0.43 (0.28 to 0.57)	0.39
Relative change post-GTN at Visit 6 (%)	8.38 (6.47 to 10.29)	9.59 (6.21 to 12.97)	0.62
Relative change **compared to baseline** post-GTN at Visit 6 (%)	-0.04 (-0.33 to 0.25)	0.01 (-0.61 to 0.62)	0.80

Table 38 – Baseline vessel diameter and percentage change throughout study (GTN)

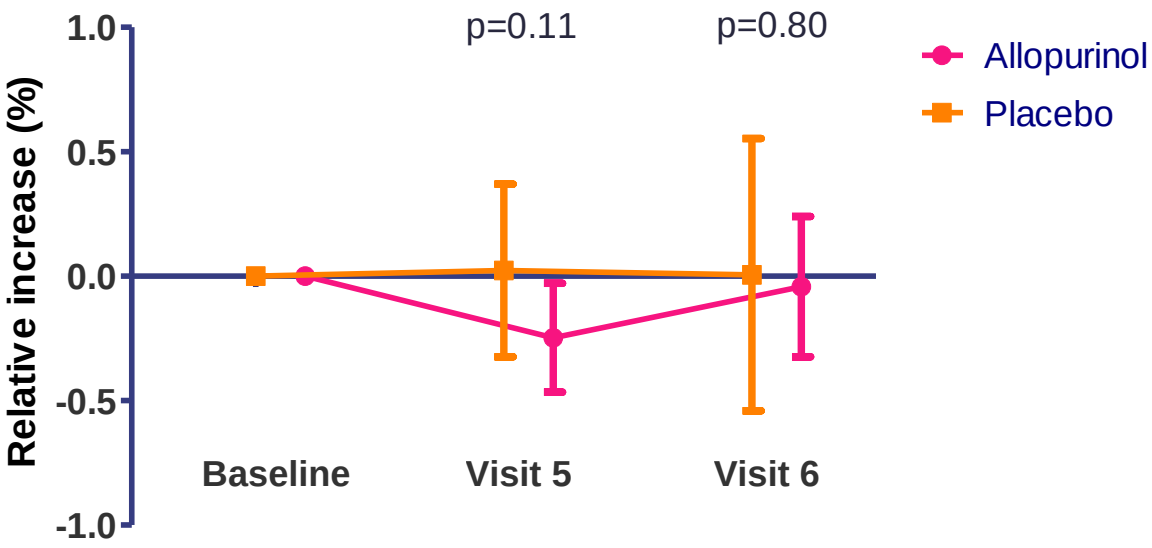

Figure 54 – Relative change in vessel diameter throughout study (GTN)

The other parameter assessed during the cuff inflation/deflation was the change in brachial artery blood flow – this was through assessment of the velocity-time integral (VTI) of the flow measured via pulse-wave doppler.

Comparison of these VTIs is shown in Table 39 (comparison between allopurinol and placebo at start and end of study), Table 40 (comparison between start and end of study by treatment group) and illustrated in Figure 55 below.

	Allopurinol Visit 2 (95% CI)	**Placebo Visit 2 (95% CI)**	**p**	**Allopurinol Visit 6 (95% CI)**	**Placebo Visit 6 (95% CI)**	**p**
Pre-cuff VTI (m)	0.18 (0.13 to 0.22)	0.17 (0.13 to 0.20)	0.78	0.15 (0.11 to 0.19)	0.15 (0.11 to 0.19)	0.99
Post-cuff VTI (m)	0.54 (0.38 to 0.70)	0.51 (0.40 to 0.62)	0.96	0.41 (0.33 to 0.50)	0.44 (0.33 to 0.55)	0.71

Table 39 – Comparison of brachial artery VTI (allopurinol vs placebo)

	Allopurinol Visit 2 (95% CI)	Allopurinol Visit 6 (95% CI)	p	Placebo Visit 2 (95% CI)	Placebo Visit 6 (95% CI)	p
Pre-cuff VTI (m)	0.18 (0.13 to 0.22)	0.15 (0.11 to 0.19)	0.34	0.17 (0.13 to 0.20)	0.15 (0.11 to 0.19)	0.80
Post-cuff VTI (m)	0.54 (0.38 to 0.70)	0.41 (0.33 to 0.50)	0.27	0.51 (0.40 to 0.62)	0.44 (0.33 to 0.55)	0.58

Table 40 – Comparison of brachial artery VTI (start vs end of study)

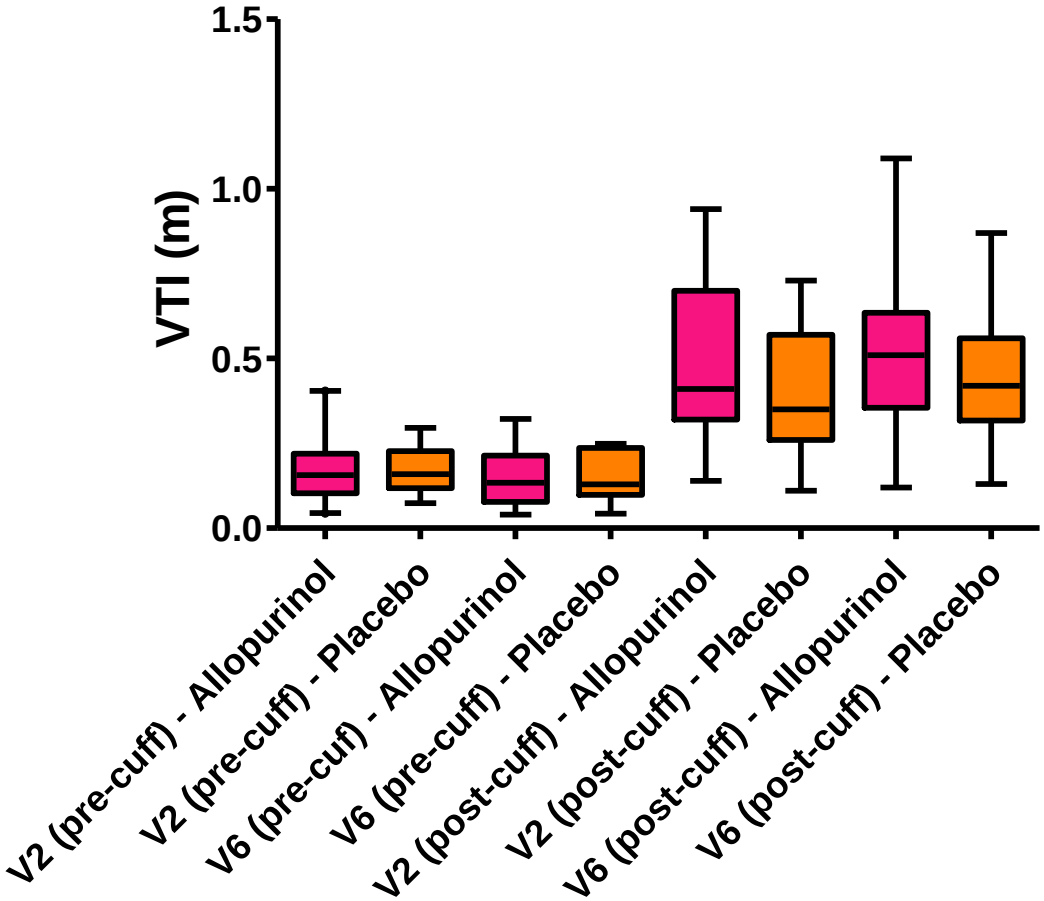

Figure 55 – Comparison of brachial artery VTI

3.9 ABI

As noted in Table 7, the baseline ABI on the affected leg was similar for both groups. Table 41 outlines the 95% confidence intervals for the values and compares allopurinol with placebo at both start and end of study. Table 42 compares the values at the start and end, splitting by treatment group. The data is represented graphically in Figure 56.

	Allopurinol Visit 1 (95% CI)	**Placebo Visit 1 (95% CI)**	**p**	**Allopurinol Visit 6 (95% CI)**	**Placebo Visit 6 (95% CI)**	**p**
ABI (ratio)	0.61 (0.56 to 0.66)	0.60 (0.55 to 0.65)	0.65	0.63 (0.58 to 0.68)	0.63 (0.57 to 0.69)	0.93

Table 41 – ABI measures (allopurinol vs placebo)

	Allopurinol Visit 1 (95% CI)	**Allopurinol Visit 6 (95% CI)**	**p**	**Placebo Visit 1 (95% CI)**	**Placebo Visit 6 (95% CI)**	**p**
ABI (ratio)	0.61 (0.56 to 0.66)	0.63 (0.58 to 0.68)	0.67	0.60 (0.55 to 0.65)	0.63 (0.57 to 0.69)	0.39

Table 42 – ABI measures (start vs end of study)

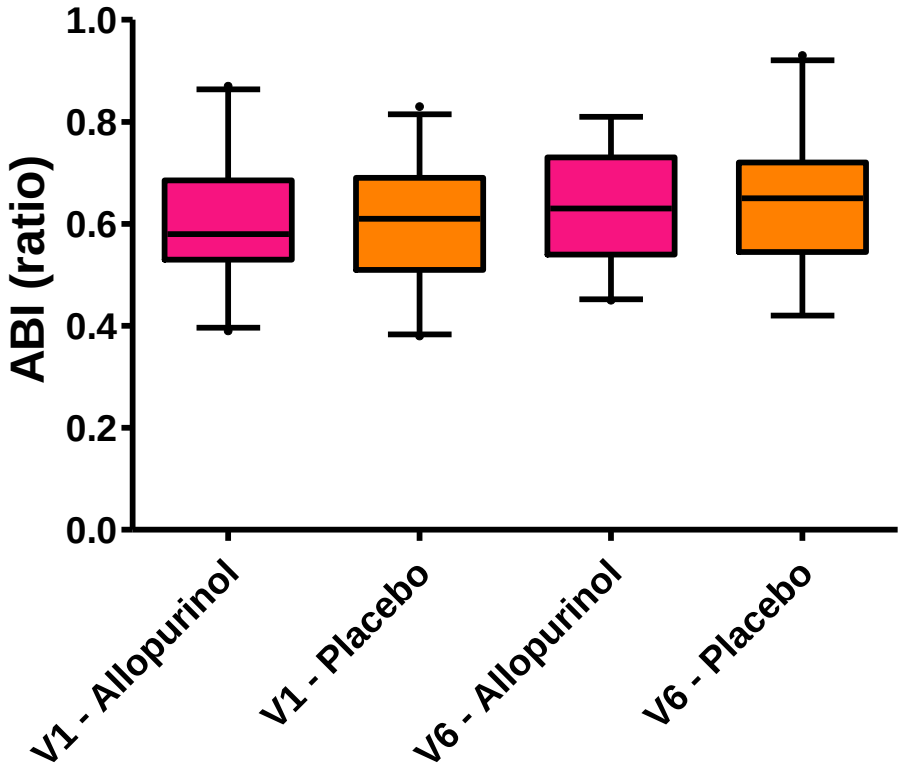

Figure 56 – ABI measures

3.10 Walking Impairment Questionnaire

As outlined in section 2.8.4, a Walking Impairment Questionnaire was administered at Visit 2 (second screening visit), Visit 4 (mid-point) and Visit 6 (final study visit).

As well an overall score the WIQ results may be broken down into the three domains of distance, speed and climb, as showing in the following sections. Lower scores correspond to poor walking distance/speed and increased pain.

3.10.1 Overall WIQ scores

WIQ scores were initially compared at each stage to check for significant differences between treatment and placebo groups.

Time point	Allopurinol (95% CI)	Placebo (95% CI)	P (between group comparison at each time point)
Baseline (score)	132.9 (108.7 to 157.1)	140.0 (111.1 to 168.8)	0.92
Visit 4 (score)	163.0 (131.5 to 194.6)	166.3 (137.4 to 195.3)	0.09
Visit 6 (score)	139.7 (96.2 to 183.2)	146.1 (111.8 to 180.3)	0.34

Table 43 – Overall WIQ scores at different study stages

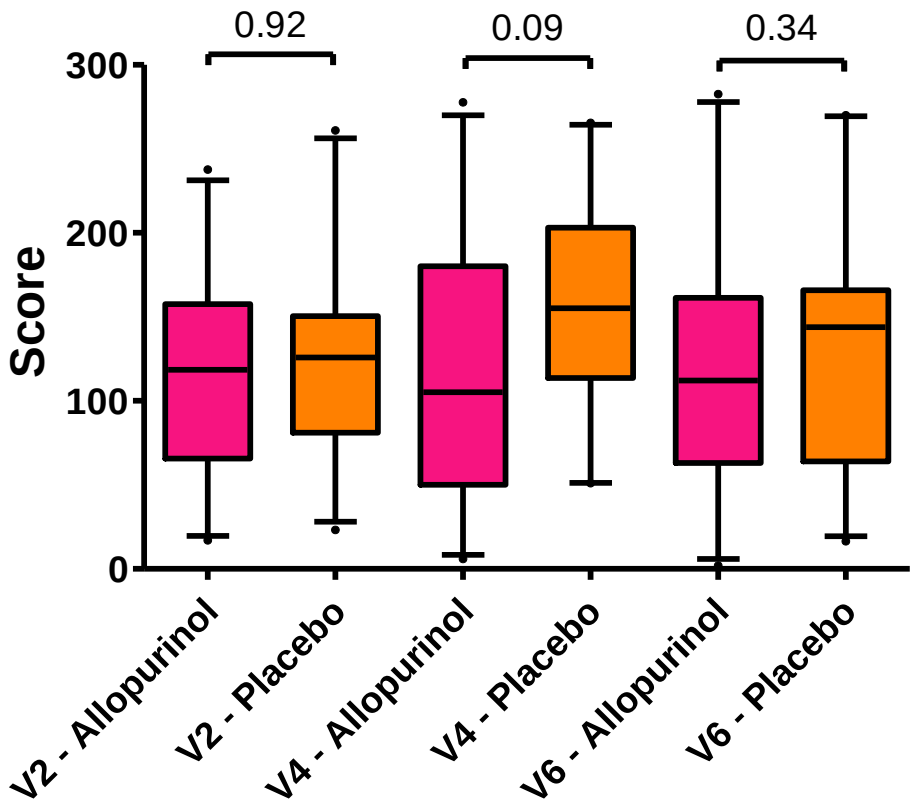

Figure 57 – Overall WIQ scores at different study stages

The difference in scores between start, middle and end of the study for both groups was then considered, as shown in Table 44.

Time point	Baseline (95% CI)	Visit 4 (95% CI)	Visit 6 (95% CI)	P (within group comparison at across the study period)
Allopurinol (score)	132.9 (108.7 to 157.1)	163.0 (131.5 to 194.6)	139.7 (96.2 to 183.2)	0.92
Placebo (score)	140.0 (111.1 to 168.8)	166.3 (137.4 to 195.3)	146.1 (111.8 to 180.3)	0.13

Table 44 – Comparison between overall WIQ scores across study stages

3.10.2 WIQ distance scores

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline (score)	41.6 (29.4 to 53.8)	46.0 (33.7 to 58.3)	0.26
Visit 4 (score)	52.8 (38.8 to 66.8)	54.9 (43.7 to 66.0)	0.40
Visit 6 (score)	39.6 (22.3 to 57.0)	47.0 (33.4 to 60.7)	0.32

Table 45 – WIQ distance scores at different study stages

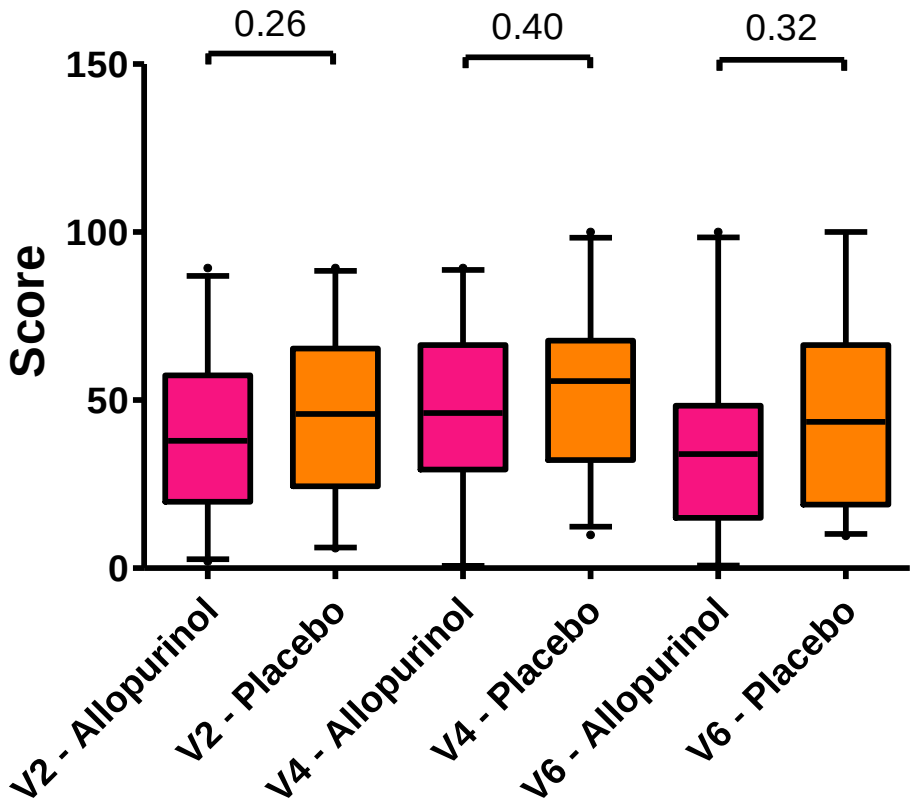

Figure 58 – WIQ distance scores at different study stages

3.10.3 WIQ speed scores

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline (score)	37.1 (29.4 to 44.8)	39.8 (30.0 to 49.5)	0.75
Visit 4 (score)	43.8 (31.0 to 56.6)	51.1 (39.8 to 62.4)	0.26
Visit 6 (score)	41.2 (26.2 to 55.1)	41.8 (30.1 to 53.4)	0.78

Table 46 – WIQ speed scores at different study stages

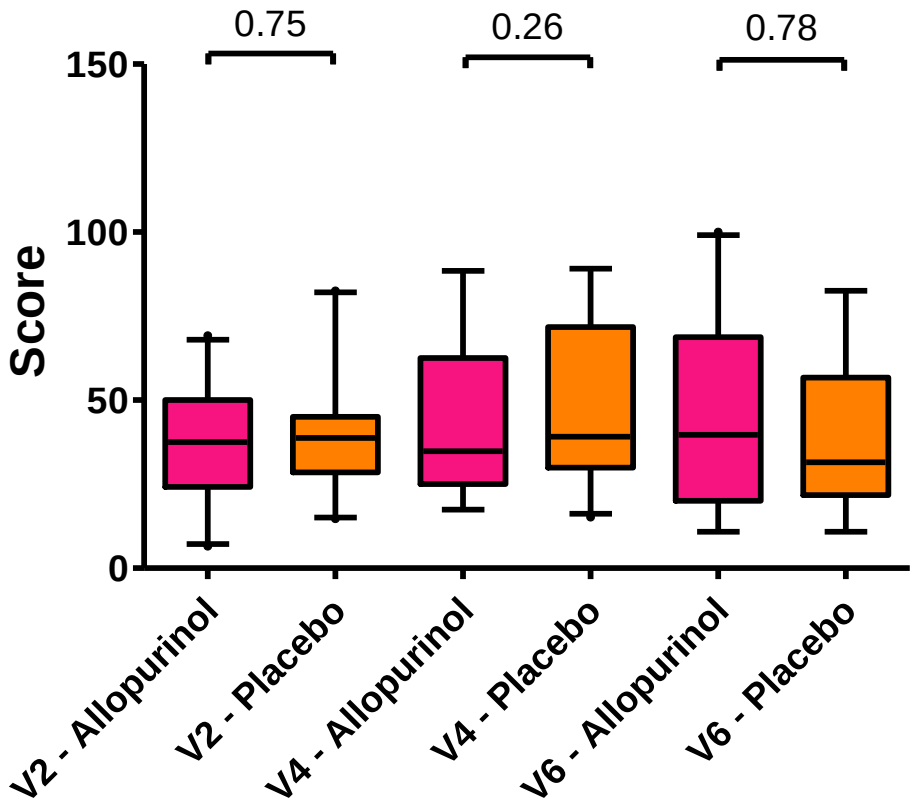

Figure 59 – WIQ distance scores at different study stages

3.10.4 WIQ climb scores

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline (score)	54.2 (43.0 to 65.3)	54.2 (41.6 to 66.7)	0.98
Visit 4 (score)	66.4 (54.4 to 78.4)	60.4 (50.0 to 70.9)	0.69
Visit 6 (score)	58.9 (43.4 to 74.4)	57.2 (44.5 to 70.0)	0.89

Table 47 – WIQ climb scores at different study stages

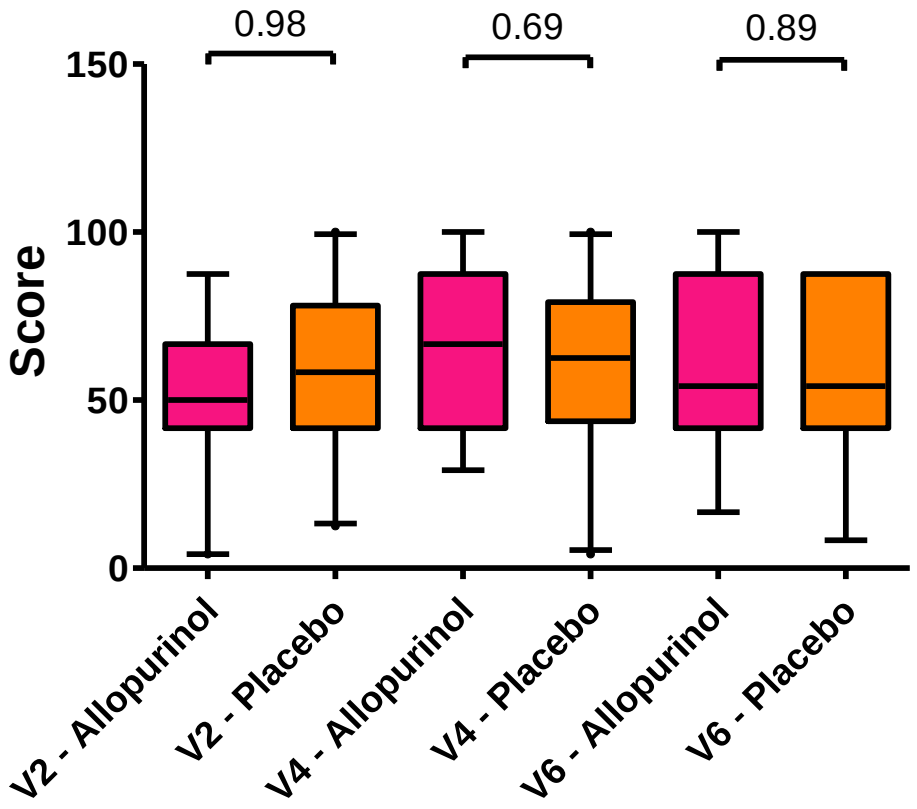

Figure 60 – WIQ distance scores at different study stages

3.11 SF-36 Quality of Life Questionnaire

As outlined in section 2.8.5, an SF-36 questionnaire to assess quality of life was administered at Visit 2 (second screening visit), Visit 4 (mid-point) and Visit 6 (final study visit).

The SF-36 questionnaire consists of scores in a number of domains:

- Physical functioning score
- Role functioning physical score
- Role functioning emotional score
- Energy fatigue score
- Emotional well-being score
- Social functioning score
- Pain score
- General health score
- Health change score

3.11.1 Overall SF-36 scores

SF-36 scores were initially compared at each stage to check for significant differences between treatment and placebo groups.

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline (score)	535.1 (457.7 to 612.5)	571.0 (519.5 to 622.4)	0.73
Visit 4 (score)	525.0 (439.0 to 610.9)	552.1 (472.9 to 631.3)	0.62
Visit 6 (score)	555.2 (485.0 to 625.5)	536.5 (470.8 to 602.2)	0.57

Table 48 – Overall SF-36 scores at different study stages

No significant change across visits was noted (p=0.92 for allopurinol; p=0.70 for placebo)

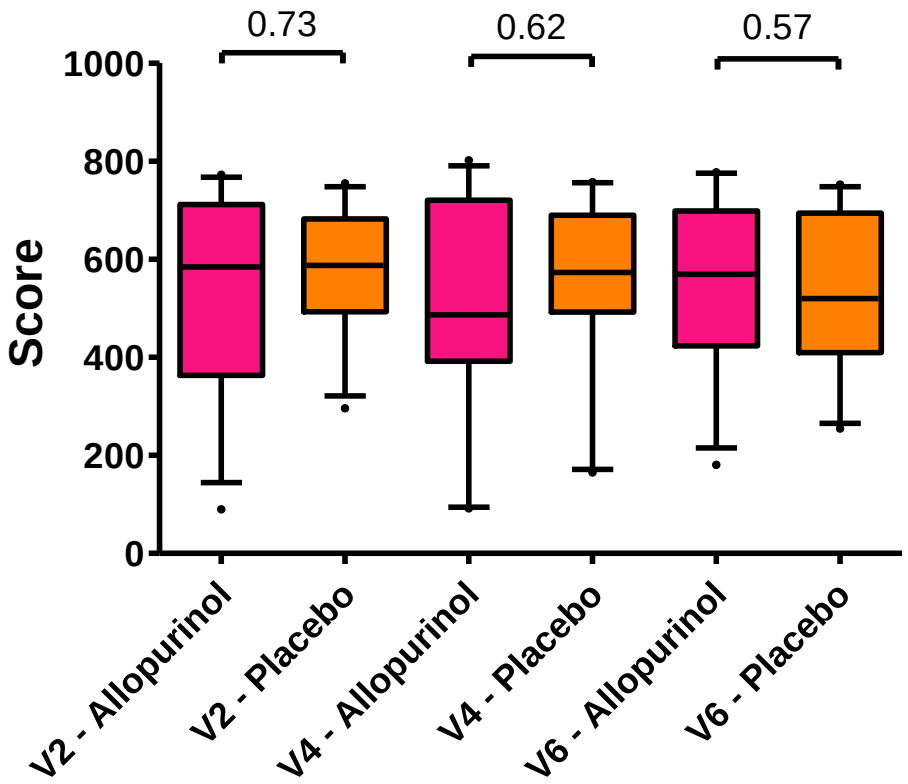

Figure 61 – Overall SF-36 scores at different study stages

3.11.2 SF-36 domains

The various domains of the SF-36 questionnaire were then considered separately.

Domain	Time point	Allopurinol (SD)	Placebo (SD)	P
Physical functioning score	Baseline	49.7 (20.7)	52.8 (17.3)	0.98
	Visit 4	48.2 (21.5)	49.8 (18.3)	0.89
	Visit 6	53.4 (21.4)	45.7 (18.8)	0.12
Role functioning physical score	Baseline	60.0 (43.3)	56.0 (39.7)	0.70
	Visit 4	52.2 (46.4)	53.4 (46.4)	0.81
	Visit 6	48.9 (43.0)	47.0 (40.9)	0.91
Role functioning emotional score	Baseline	74.7 (38.8)	84.0 (34.9)	0.26
	Visit 4	75.4 (40.4)	69.7 (41.0)	0.56
	Visit 6	80.4 (33.6)	78.8 (35.0)	0.89
Energy fatigue score	Baseline	55.0 (23.0)	59.5 (18.6)	0.67
	Visit 4	52.4 (26.4)	57.3 (19.4)	0.62
	Visit 6	60.7 (19.0)	53.2 (22.8)	0.34
Emotional well being score	Baseline	71.2 (26.3)	79.2 (19.1)	0.21
	Visit 4	70.6 (30.9)	79.8 (18.8)	0.35
	Visit 6	80.7 (13.1)	76.0 (24.4)	0.95
Social functioning score	Baseline	71.3 (30.0)	86.7 (17.8)	0.06
	Visit 4	71.8 (31.3)	82.2 (21.6)	0.40
	Visit 6	76.5 (24.4)	80.9 (24.3)	0.47

(continued on next page)

Domain	Time point	Allopurinol (SD)	Placebo (SD)	P
Pain score	Baseline	59.0 (26.6)	56.8 (21.8)	0.63
	Visit 4	56.3 (31.8)	56.6 (24.5)	0.98
	Visit 6	53.7 (28.7)	56.1 (21.1)	0.80
General health score	Baseline	49.2 (26.1)	51.0 (21.8)	0.96
	Visit 4	51.3 (27.0)	54.5 (23.8)	0.68
	Visit 6	57.4 (19.2)	54.5 (24.7)	0.85
Health change score	Baseline	45.0 (17.7)	45.0 (17.7)	1.00
	Visit 4	46.7 (11.4)	48.9 (18.1)	0.48
	Visit 6	43.5 (15.5)	44.3 (13.2)	0.99

Table 49 – SF-36 scores by visit and domain

3.12 Oxidised LDL

Oxidised LDL levels were measured both at baseline (Visit 1), midpoint (Visit 3) and end of study (Visit 6). The results were as noted in Table 50 and Figure 62.

Time point	Allopurinol (95% CI)	Placebo (95% CI)	p
Baseline (U/l)	53.0 (45.7 to 60.3)	49.4 (43.1 to 55.8)	0.62
Visit 3 (U/l)	45.2 (39.1 to 51.3)	49.4 (41.9 to 57.0)	0.46
Visit 6 (U/l)	57.2 (44.8 to 69.6)	45.8 (39.2 to 52.4)	0.21

Table 50 – Oxidised LDL results by visit

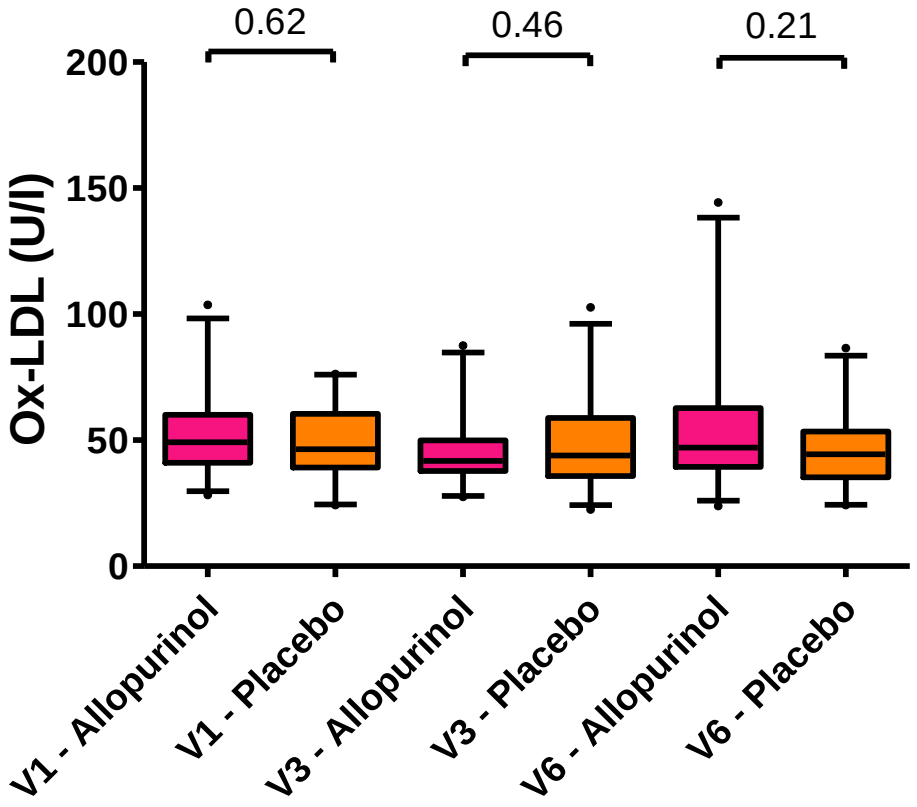

Figure 62 – Oxidised LDL results by visit

Difference in scores between start, middle and end of the study for both groups was then considered, as shown in Table 51.

Time point	Baseline (95% CI)	Visit 3 (95% CI)	Visit 6 (95% CI)	p
Allopurinol (U/l)	53.0 (45.7 to 60.3)	45.2 (39.1 to 51.3)	57.2 (44.8 to 69.6)	0.15
Placebo (U/l)	49.4 (43.1 to 55.8)	49.4 (41.9 to 57.0)	45.8 (39.2 to 52.4)	0.66

Table 51 – Oxidised LDL results by treatment group

4 Discussion

This double-blind, placebo controlled, parallel group study aimed to evaluate whether treatment with allopurinol for six months would prolong exercise duration in patients with PAD. In this section the findings of the study (already presented in the results section) will be discussed and the possible underlying mechanisms for these findings, along with the strengths and limitations of this study, will be considered.

4.1 Primary outcome – treadmill testing

The results of the treadmill testing are laid out in Section 3.5. As explained in the methods, there are two key items that are conventionally measured when carrying out a treadmill test – claudication onset distance and peak walking distance. In both of these there was a slight trend to higher baseline values in the placebo group (despite the significantly higher pack year history in this group), however these did not reach statistical significance. By the end of the study both measures had increased in each arm of the study – this was of the order of 40m for COD and 27m for PWD, again with no significant difference between active and placebo groups, although if anything the increase in the placebo groups was higher. This increase over time in walking distance in the placebo arm is something that has been recognised previously in studies of PAD and will be discussed further below.[223]

To see if there was any sub-group that was showing benefit from allopurinol the treadmill results were then split into those with high/low baseline COD/PWD and those with high/low baseline uric acid/systolic BP and ABI. None of these subdivisions made any change to the results and there remained no statistically significant difference between the two groups following six months of treatment. It should however be emphasised that the study was not powered to evaluate sub-groups, as this meant comparisons were between very small numbers (12-13 participants per group). These sub-group analyses have been included to help demonstrate that the data has been fully explored and that, as such, any small potential benefit had not been overlooked – had there been any positive results from the sub-groups these

would only have been able to have been taken as an indication of potential than a firm result.

The reasons for why allopurinol should potentially be of benefit in PAD were discussed in the introduction – in particular Section 1.4 outlined the other disease processes in which it has been shown to be useful. In this study, despite a clear reduction in uric acid in the group treated with allopurinol, there has been no evidence of any beneficial effect on walking distance when on the treadmill. This may be due to problems with treadmill testing as an outcome measure (i.e. there is an effect but it is not detecting it), or it may be that PAD is a different disease process that is not amenable to improvement with allopurinol. Each of these possible explanations will now be considered in turn.

4.1.1 Treadmill testing as an outcome measure

It has been previously recognised that there are difficulties with monitoring treadmill performance over time due to increases in maximal walking distance following repeated testing. This is particularly the case with constant-load treadmill protocols, where both speed and grade remain constant throughout the test – the co-efficient of variation on this type of test can be almost 45%. Switching to a graded approach (where speed is constant but the grade gradually increases) can reduce the co-efficient of variation to 15-25%, although it does still remain an issue.[20]

This improvement in exercise distance over time in patients not on active therapy limits the ability to assess treatment effect in study subjects. The mechanism

underlying this effect is unknown but may represent a "learning curve" as patients repeat the test, a classic placebo effect, or it may be due to improved walking biomechanics as subjects become familiar with the treadmill apparatus.[20] It has also been previously reported that the placebo effect is particularly strong in conditions where pain is a major factor, as is the case with PAD, therefore an endpoint that relates to pain is more likely to be influenced by placebo effect.[223]

Evidence to date suggests that although both COD and PWD should be recorded it is the latter that has been found to be more reproducible. This is perhaps counter to the patient's perspective – they are probably more concerned about an increase in COD following treatment as this will have greater impact on the range of activities that they can undertake with minimal symptoms. Most patients will continue to walk after appearance of the first signs of pain (COD), but few will walk until their maximum pain threshold (PWD) is reached during the course of daily activities.[224] In practical terms however many study participants had difficulty with knowing when to indicate they had developed pain – some felt an occasional early twinge that settled briefly before returning later, many forgot to mention it at all without repeated prompting as it was just something they were so accustomed to in day-to-day life.

An interesting study conducted by Gardner *et al* in 1991 looked at the effect that handrail support had on patients with PAD undergoing treadmill testing.[225] They found that both COD and PWD were significantly greater when patients were allowed to use the handrails for support. The magnitude of increase was around 10m in COD and almost 75m in PWD. They also found that these distances tended to increase upon

repeated testing when handrail support was allowed. There were no significant changes in foot transcutaneous oxygen tension and ABIs following exercise to maximum tolerable pain, regardless of handrail support. They concluded that due to the alterations in claudication distances handrail support should not be allowed when assessing patients with PAD, unless balance cannot otherwise be maintained. In this study there was no strict policy regarding use of the handrails. Some patients hardly used them, others did utilise them. If asked they were advised to use them as required but their usage was not recorded. In retrospect it may have aided consistency to have tried to minimise handrail use except for those patients who really required it. Nicolaï *et al* also confirmed that walking distances were greater when a steady speed/progressive incline protocol was used, as opposed to a single-stage protocol. This is something that has been previously recognised and was the reason for the Gardner protocol being used in this study.[224]

Treadmill testing was however not the only method of assessment used in this project. Zwierska *et al* showed in 2004 that shuttle walk testing exhibits similar test-retest reliability as treadmill testing, but that it evoked a lower level of cardiovascular stress and is preferred to treadmill testing by a large proportion of patients.[226] Based on previous usage in patients with PAD it was decided that a variation on this (the 6MWT) would be used in this study.[207] Qualitative feedback from patients comparing the 6MWT and the treadmill test indicated that the majority preferred the 6MWT and found it to be more akin to their normal day-to-day routine (especially being allowed to stop if required), however some preferred the physical support of the treadmill machine and the handrails. Given the variable walking ability of the patients in the

study and the aforementioned problems with treadmill testing (despite its wide usage in PAD studies) it was useful to have made use of both methods of assessment.

4.1.2 PAD as a different disease process

We know that allopurinol has been shown to be of benefit in angina, in some ways a similar disease process, yet in this study it has not been of benefit in patients with PAD. Rekhraj *et al* showed that allopurinol reduced LV end systolic volume and LV afterload (as measured by augmentation index) in patients with ischaemic heart disease, which suggests that offloading the LV may be another mechanism contributing to the anti-ischaemic effect of allopurinol in angina pectoris.[188] This possibility is highly relevant to the work in this thesis since this particular effect on LV afterload would produce an anti-ischaemic effect on the heart but not an anti-ischaemic effect in PAD.

As discussed earlier, oxidative stress is part of the disease process of PAD. Edwards *et al* found that exercise in patients with PAD leads to a neutrophilia and thromboxane production with subsequent endothelial injury. The antioxidant activity of glutathione peroxidase was also reduced in patients with claudication and this may allow the unopposed action of free radicals to damage endothelium.[227] One would therefore hope that helping to address this oxidative stress with allopurinol would be of benefit. Indeed Mellin *et al*. showed a reduction of ROS, using electron spin resonance spectroscopy, acutely after initiation of allopurinol. However long-term treatment did not sustain this reduction.[156] This lack of long-term benefit may be due to the breakthrough of other sources of ROS production such as NADPH oxidase and may be a potential reason for the lack of symptomatic benefit.[130, 159, 161] Gresele *et al* made a

similar attempt to offset the effects of ROS, this time by increasing the levels of circulating NO via an NO-donating agent (NCX 4016) – they found no improvement in PWD.[228]

A small (12 patient) study of young asymptomatic individuals with familial hypercholesterolemia found endothelial XO activity to be increased by >200% compared with age-matched controls, suggesting that activation of vascular XO may represent an early mechanism contributing to increased radical formation and endothelial dysfunction in the atherosclerotic disease process.[127] This may indicate that XO blockade may have a role earlier in the disease process, however by the time the disease has become established (such as in the PAD patients in this study) it is less likely to have a beneficial effect.

With PAD we are also dealing with a different calibre of vessel. The coronary arteries at their largest (the left main stem) measure 4.5±0.5mm, falling to 1.9±0.4mm in the distal left anterior descending artery.[229] By comparison the peripheral arteries in the legs are more than twice this diameter – with the common femoral artery having a diameter of 9.3±1.1mm, falling to 4.9±0.6mm when it reaches the distal popliteal artery.[230] Therefore when looking at the absolute level of increase in vessel compliance that may be possible through improving oxidative stress (as shown in the positive effects of allopurinol on FMD in many other studies) it can be seen that this is likely to produce less of a relative increase in vessel diameter (and thus potential blood flow) in these larger arteries. This may also be why endovascular approaches to treatment of PAD have been less successful than similar approaches in the smaller

calibre coronary arteries, where stenting has been highly efficacious. It is well recognised in coronary angioplasty that an underexpanded stent is more likely to restenose – with the size of peripheral vessels being considered in PAD it may be difficult to ensure adequate expansion, where in general there has been less use of intravascular ultrasound techniques. There are newer imaging techniques, such as optical coherence tomography, which offer enhanced imaging resolution and contrast that may prove of help in this area.[231] The additional information provided via these imaging modalities has provided useful further information about the progression of atherosclerosis. In particular it appears that the decrease in luminal area cannot be attributed to solely the increase in plaque – arterial wall shrinkage (mainly through loss of the internal elastic lamina) is a paradoxical mechanism that may contribute to the severe luminal narrowing of the atherosclerotic femoral artery.[232] Imaging has also helped with the development of new balloon technologies, such as 'cutting balloons' that have proved helpful in improving outcomes in cases of restenosis.[233]

When one considers other drugs that are of benefit in cardiac disease it can be seen that many of them (with the exception of anti-platelet agents) have not been shown to have been of benefit in PAD. For example vasodilators were initially hoped to be a useful treatment for claudication symptoms, yet repeated studies have found no benefit for clinical efficacy. In an excellent review article on the topic of pharmacotherapy in PAD, Hiatt explains the likely pathophysiology for this. It is thought likely to be because during exercise, the portion of a resistance vessel located distally to a stenosis or occlusion dilates in response to ischaemia. Vasodilators do not affect these vessels, whose dilation is due to endogenous factors, but they may

decrease resistance in other vessels, leading to a “steal” of blood flow away from the under-perfused muscle. Vasodilators can also lower systemic pressure, leading to a reduction in perfusion pressure.[48]

4.2 Secondary outcomes

4.2.1 Six Minute Walk Test

Baseline distances walked during the 6MWT were very similar for both groups (mean 401.2m allopurinol vs 389.1m placebo, p=0.72). There was no meaningful change in these levels throughout the study period. Given the variable baseline walks for each participant the mean absolute and relative change at mid-point and end of study was also calculated. This showed a very small trend towards a 'learning effect' (as discussed further under strengths and limitations in section 4.3), but in practical terms there was no real change, certainly not of the 30m or so level that would be considered clinically meaningful (and that the study was therefore powered to detect).

The 6MWT results were then sub-divided in two further ways – by baseline walk and uric acid. The first split participants along the median baseline walk to see if any effect was restricted to good or poor walkers – this made no significant change to the results. For uric acid both median baseline uric acid level and those with high/low percentage reduction in uric acid were considered – again this did not change the results and there remained no clinically meaningful change in distance walked.

The underlying reasons for this lack of response on 6MWT are likely similar to those discussed in the previous section for treadmill testing. Given the different nature of the stress provided by this test compared to treadmill testing it was still something that was worth assessing – indeed many study participants remarked that they felt this test was a better reflection of their 'real world' walking pattern, particularly with the

ability to stop and take a break if required. There is some evidence that 6MWT performance is more closely correlated with physical activity during daily life than treadmill measures.[234] It also remains a useful test in a prognostic sense across a wide variety of disease processes and is one that is easily tolerated by patients with a number of exercise-limiting conditions.[235-239]

As mentioned in the previous section an alternative to the 6MWT is the shuttle walk test (SWT). In this test an audio tone sounds at a pre-defined interval – the participant has to reach the cone by the time the tone sounds. This has a similar advantage to the 6MWT in that it provides a challenge closer to the patient's usual way of walking than that observed with the treadmill, but has the advantage of having the patient's speed controlled externally, allowing for a progressive increase of the walking effort and thus perhaps better potential for revealing the patient's functional capacity. However, traditionally the SWT has not been widely utilised with PAD patients, and its psychometric characteristics (given the need to reach a target on every lap and the sense of failing against a standard rather than just being asked to do as much as they can manage) still need to be evaluated with this population and contrasted with different protocols concerning functional assessment.[219]

4.2.2 Blood pressure

As mentioned in Section 3.7, BP had not been identified as a key secondary outcome measure for the study. Therefore although baseline values were collected as part of the initial physical examination the subsequent readings were taken prior to 6MWT exercise and thus (due to the organisation of the visits) this was mainly following

administration of GTN spray for FMD. Given the effect this has on BP it was therefore only possible to compare post-GTN readings between Visit 2 and Visit 6. There was no significant change noted between these visits – however the level of reduction normally noted by treatment with allopurinol is likely to have been masked by the magnitude of drop in BP following GTN administration. Comparing the mean BP in the baseline characteristics (i.e. without GTN) with the post-FMD result shows a reduction from 155/78mmHg to 120/69mmHg. By comparison with allopurinol the magnitude of reduction that would be expected would be of the order of 3/1mmHg.[170] This small change will easily be obscured by the much more marked effect of GTN administration and thus it is not possible to properly comment in this study on any effect on participants' blood pressure from treatment with allopurinol. Nevertheless the vast majority of studies of allopurinol found no significant effect of allopurinol on BP.

4.2.3 FMD

The results of FMD testing are shown in Section 3.8. It can be seen that the baseline values were similar in both active and placebo groups. The relative change in vessel diameter following hyperaemia (i.e. post BP cuff deflation) was very slightly better in the allopurinol group, however the absolute level was still very small (<0.5%) and the difference between active and placebo groups was not statistically significant.

Following administration of GTN there was a single result that was statistically significant (visit 5), however this was only for the relative change within that visit – when compared to baseline it was no longer significant.

There was no significant change in brachial artery blood flow (as measured by VTI) in either group following treatment.

Although these findings are in-keeping with other outcome measures in this trial, a number of other studies of allopurinol have previously shown an improvement in FMD. For example, Rekhraj *et al* showed an improvement following allopurinol treatment of +0.82±1.8% vs. placebo -0.69±2.8%; p=0.017.[188] This was after nine rather than six months of treatment, however even looking at six months in their study, there was a clear separation between the two groups. The magnitude of the change was not that much greater than that seen here, however the spread of results was much tighter.

One other recent study of allopurinol found no effect on endothelial function. Szwejkowski *et al* postulated that the lack of effect on FMD results in their study may be that their patients with type II diabetes mellitus were very well treated with statins, ACE inhibitors and ARB's which are each known to improve endothelial function. They went on to explain that this was evident in the baseline measures of vascular health in their patients – the baseline augmentation index was only 11% therefore potentially making it difficult to improve it further with allopurinol.[190] Augmentation index was not a measure that we elected to use in this study.

FMD is a useful non-invasive test in patients at risk of cardiovascular disease. Muiesan *et al* showed that in hypertensive patients FMD (but not endothelium-independent dilatation by way of GTN) helped identify those at risk of non-fatal and fatal cardiovascular events.[240] However as clearly outlined by Corretti *et al*, it is a test that

can be influenced by many factors and the results can be confounded by these factors.[173] For example in ideal conditions they explain that all vasoactive medications should be withheld for at least four half-lives. It could however be argued that as we were looking at allopurinol as an add-on therapy it would not have been appropriate to have done this as it would not have represented a fair comparison against current medical treatment. It is also advised that subjects should fast at least eight to 12 hours before the study, should not exercise, or ingest substances that might affect FMD such as caffeine, high-fat foods and vitamin C or use tobacco for at least four to six hours before the study. In practical terms with my study population it was not always possible to reliably take account of these factors. In particular some patients could not easily attend morning appointments and those who continued to smoke were not keen to abstain from doing so prior to study visits, or did so intermittently. These factors may have combined to lessen the effect of any change in FMD readings. There is certainly good evidence from invasive testing of the role that cigarette smoking has on impairing vascular function.[241]

It also has to be considered that the patient population being studied here, all with proven PAD, had stiffer vessels that were less likely to respond to treatment.[242-244] Interestingly in patients with end-stage renal disease (another group with more calcified and less-compliant arteries) there was also less evidence of utility of FMD measurement by way of prognostic value.[245] A study by Payvandi *et al* showed that in PAD patients undertaking exercise, those with higher levels of physical activity had a greater percentage change in brachial artery FMD.[243] This was an observational study

but it could mean that those able to maintain higher levels of activity are patients with milder disease.

4.2.4 ABI

No significant change in ABI was noted between the start and end of the study in the two groups – the mean ratio remained approximately 0.61. This is consistent with the lack of clinical response to treatment.

When considering alternative treatments for PAD, a small number of studies using cilostazol have compared ABI pre and post treatment. The weighted mean difference for the mean change in ABI was in favour of treatment with cilostazol to a small degree (WMD 0.06; 95% CI: 0.03 to 0.09) compared with placebo.[67] A Cochrane review of numerous studies of pentoxifylline found none that reported any significant change in ABI following treatment – at most there was a very small non-significant trend to improvement, but in many no change was reported.[72]

There are some issues with the standardisation of measurement of ABIs between centres, although this was not something that impacted on this study given all measurements were carried out by AR.[246] It could however be argued that repeated measurements would have been valuable in ensuring maximum accuracy.[247] ABI remains a valuable tool in both initial assessment of patients with PAD and in a prognostic sense with regards future cardiovascular events.[245, 248]

4.2.5 Questionnaires

There were no significant changes in either the general quality of life questionnaire (SF-36) or the disease-specific Walking Impairment Questionnaire. This is in-keeping with the lack of difference in either COD or PWD between treatment and placebo groups.

The WIQ has been well-validated in the PAD population, in particular there is good correlation between claudication onset/peak walking distances and WIQ pain, speed, and distance subscales. Overall the WIQ is said to be the most specific questionnaire for documenting the qualitative deficits of the patient with claudication while providing strong relationships with the quantitative measures of arterial disease.[249] It was therefore an appropriate choice of questionnaire in this study and the lack of improvement in scores for those participants receiving allopurinol is unsurprising given the lack of change in the primary outcome. Indeed if there had been a positive result in this area it would have been surprising given the known correlation with objective measures.

SF-36 is a much more general (albeit widely utilised) questionnaire and again the lack of improvement in scores here is not surprising given the lack in change in objective measures. In most domains there was a trend to slightly higher scores in the placebo group at baseline, however none of the differences reached statistical significance and this is likely just a consequence of the slightly small sample size involved. There were no significant changes following treatment.

4.2.6 Oxidised LDL

Evaluation of oxidative stress is a difficult task *in vivo* due to the very short half-life of ROS. However the consequence of ROS activity, by way of lipid peroxidation, is something that can be measured.[250-252] In this study oxidised low-density lipoprotein was chosen. No significant changes were found in Oxidised LDL levels in either treatment or placebo groups throughout the duration of the study.

However of interest is the level of Ox-LDL detected in this patient population. The mean baseline levels were in the 43-60 U/l range (51.2±16.6U/l), which seems to be quite low in comparison to some other studies. For example a population study of 35-55 year olds by Langlois *et al* looked at femoral and carotid wall damage via ultrasound imaging. When these measurements were compared with Ox-LDL levels they found that males had generally higher levels than females and that those with evidence of femoral and carotid artery damage had higher levels than those without. The Ox-LDL levels for those without evidence of wall damage were 87.9±37.2U/l (female) and 93.3±36.1U/l (male), rising to 107.3±49.3U/l (female) and 110.7±41.3U/l (male) in those with damage to both vessels.[253]

By comparison a study of 72 young (30±10 years) healthy Spanish subjects found levels of 58.4±13.6U/l (female) and 68.1±17.7U/l (male).[254] These levels appear to be more in line with the levels found in this study – this is surprising as one would have expected to have found higher levels here given the degree of arterial damage evident. However another comparator is the baseline levels in patients with type II diabetes and left ventricular hypertrophy in the study by Szwejkowski *et al* – these were in the

range of 28.5±9.9U/l and did not significantly change following treatment with allopurinol.[190] Kao *et al* had similar baseline values of 31.1±8.85U/l in their study of patients with chronic kidney disease and found a trend towards slight improvement in the active group, although it did not reach statistical significance.[189] A study of 107 healthy adults stratified by uric acid levels showed a trend towards increased Ox-LDL levels with increasing serum uric acid levels (from 55.7±16.0U/l [1st quartile, UA <5.3mg/dl] to 60.3±18.2U/l [4th quartile, UA>7.0mg/dl]) but these differences were not found to be statistically significant.[179]

This marked variability in levels between different studies may be related to the levels of disease activity but could also be due to the technical differences in measuring Ox-LDL levels between different laboratories and different assays, as discussed further below. The comparators from Szwejkowski and Kao were both analysed using the same process/equipment as in this study.

Much of the published work in PAD appear to look more at anti-Ox-LDL antibody levels that than Ox-LDL levels themselves, however one study from Andican *et al* in 2008 did measure Ox-LDL. They considered 42 patients, half with angiographically-proven PAD and half as 'healthy controls'. The Ox-LDL levels in the control group were 72.2±20.1U/l and in the PAD group were 87.0±24.7U/l.[255] The higher levels in their PAD group were statistically significant, however it should also be noted that there was an almost 15 year mean age difference between their control and PAD groups ($p<0.001$) and their PAD group were almost exclusively male compared to a 50/50 split in the control group ($p<0.01$) – this makes it more difficult to solely attribute the

difference to be due to PAD given the existing evidence that increasing age, cholesterol and male sex lead to higher Ox-LDL levels. Interestingly when they split their PAD group into those with mild vs severe disease by Fontaine grading there was almost no difference – 88.3±21.5U/l (mild) vs 85.5±28.8U/l (severe).

In terms of this study however, these low levels of Ox-LDL at baseline would certainly go some way to explaining the lack of response to allopurinol. Why they are so low is however unclear given the clear disease burden in the patient population. One could also postulate that given the majority of patients in this study were ex-smokers rather than active smokers their Ox-LDL levels may be slightly lower. There is also some evidence that measuring the anti-Ox-LDL antibody levels may give more stable results than the more complex process of directly measuring Ox-LDL levels.[256] However there are many different auto-antibody assays available, which can give quite varying outcomes themselves, therefore picking the correct one would be important.[257]

Although comparing different Ox-LDL values between different published studies is informative to some extent, such results should not be over-interpreted, especially between different laboratories.

4.3 Strengths & limitations

As a double-blind placebo-controlled parallel group study both the participants and investigators were blind to treatment. All data collection was carried out by a single investigator, eliminating inter-observer variability. The ongoing participation rate was good, with few patients leaving prior to completion. There was an equal balance between both arms of study with respect to withdrawals, with three in the active arm and two in the placebo group (see CONSORT diagram, Figure 20). The numbers that did leave were lower than predicted and the study appears to have been appropriately powered, with no evidence of any trend towards a positive result that was just below the level of statistical significance – it was clearly negative.

As well as higher than expected numbers remaining in the study, the level of uric acid reduction was also very good – as outlined in section 3.3 the majority of participants in the active arm experienced a 40-60% drop in uric acid levels. This is higher than the levels achieved in a number of other trials and is no doubt due to the high dose (600mg/day) of allopurinol being used.[164] This dose was well-tolerated with withdrawals from the study occurring prior to the final dose increase. At the start of the study there was noted to be a degree of confusion amongst some participants regarding the different IMP doses/bottles. A guidance sheet (Appendix F) was therefore created and this proved to be very effective in resolving the matter.

There was good use (over 90%) of anti-platelet and statin therapy in all patients prior to entry into the study, as per guidelines for management of PAD. There were very few changes to medication amongst participants during study participation. Baseline

characteristics between the groups were broadly similar, with the exception of pack years smoked – this was higher in the placebo group (49.7 years v 31.7 years in the active group). Although this difference did reach statistical significance (p=0.04) there remained a long smoking history in the placebo groups and baseline ABIs were almost identical.

The choice of primary and secondary endpoints was consistent with other major trials of PAD, with absolute walking distance evaluated by treadmill testing being the most widely utilised approach.[67] The degree of clinical improvement that was being sought was also in line with other trials of PAD treatment.[25, 67] However, as discussed above there are problems with treadmill testing – in particular it may have been beneficial to have had clear guidance regarding the use of handrails.

The study was however a single-centre concern, with relatively small numbers of participants in absolute terms. This did mean though that there was a consistency of approach in assessment of patients and a close adherence to the study protocols. Some issues only become evident after starting the study visits – for example the distance of the test rooms from the main entrance to the hospital meant that some patients were arriving with pre-existing calf muscle fatigue. Wheelchair collection from the main entrance by AJR was offered to all patients but the majority declined this. In cases where they had accepted it on the first visit they were encouraged to persist with this on subsequent visits to ensure consistency. After the first couple of patient visits the administration of questionnaires was moved to the beginning of the visit to ensure an adequate rest period prior to undertaking exercise.

As noted previously it would have been useful to have consistently recorded participants' BP at start/mid/end of study separate to the administration of GTN to allow for assessment of the response of this variable to allopurinol.

There were issues in consistently assessing the claudication onset distance upon treadmill testing given the variation between participants in their reporting of pain – some would forget to mention it and only volunteer the information upon prompting. There was also a clear heterogeneity in the participants with regards severity of PAD – some managed to walk only a minute or two on the treadmill before having to stop due to pain, yet others were able to manage a much more considerable distance. In some respects this could be seen as a limitation, however in reality it reflects the clinical spectrum of PAD. Attempts were made in the analysis to sub-divide the participants into 'poor' and 'good' walkers both in regards COD and PWD (sections 3.5.2 and 3.5.3 respectively) – it could be argued that this sub-division reduced the numbers available for analysis by too great a degree (the study was certainly not powered for such comparisons), and that greater participant numbers (or a focus on particularly good or poor walkers) could have been beneficial, however there did not appear to be any trend that would have reached statistical significance with higher numbers.

When considering baseline treadmill measurements most studies consider the mean of at least two treadmill tests. However there are varying approaches as to how stability is defined. In this study a <25% change in PWD between the two tests was the pre-

defined criterion. However Mohler *et al* (amongst others) opted for a different approach due to the heterogeneous population of PAD patients. [46] They carried out an initial screening ETT and then required patients to undergo at least two further exercise tests on different days and meet one of the following two criteria:

1. PWT of between 1 and 5 minutes in the first qualifying exercise test and PWT in the second (consecutive) test within 60 seconds of the preceding test (both must be greater than 1 minute), or
2. a PWT of between 5 and 12 minutes in the first qualifying exercise test and a PWT in the second or sequential test within 20% of the first (but less than 12 minutes). The baseline for future comparison was defined as the mean of two qualifying treadmill visits.

This is an interesting approach – on the one hand it is one extra baseline treadmill compared to the protocol used in this study and is slightly stricter on those walking >5 minutes. However for the 1-5 min walkers it is actually more generous in the degree of variation allowed. This would have made recruitment slightly easier but it could be argued that the strict relative difference approach used here should have if anything selected patients with more reproducible symptoms.

With regards the 6MWT there is an element of a 'learning effect' at play. This is something that has been recognised previously and is felt to be of the order of around 4.5% in healthy subjects, possibly higher in those with more disease burden.[258] It tends to decrease with repeated test administration, plateauing after three tests.[259] In this study only three tests were carried out (baseline/mid/end). Only a small learning effect of around 3% appeared to be present in this study but an increased number of

tests would have helped to reduce the impact of this. This would however have to be balanced by the fatigue experienced by patients – further walking tests would have either necessitated extra study visits or a much increased visit length to allow for sufficient rest, potentially reducing the acceptability of the study protocol to participants.

The statistical analyses carried out when looking at the changes in walking distances over time could also have been made more robust. In particular when taking into consideration the differing baselines in active and placebo group and the change in both over time it would have been more appropriate to use an analytical technique that took these factors into account, such as analysis of variance (ANOVA) or generalised linear mixed models (GLMM).

4.4 Conclusion

This study showed that treatment with allopurinol did not prolong exercise duration in patients with peripheral arterial disease. As discussed above there may be some issues with the reliability of treadmill testing, but the baseline stability criteria were kept quite strict to attempt to minimise this. Of particular relevance is the fact that it was not just ETT performance that did not improve – all other measures of exercise, vascular health and indeed the participants' own assessment of their health by way of questionnaires did not change.

In view of the excellent reduction in uric acid levels in the allopurinol group this would suggest that it is the treatment itself that does not work in this disease process rather than a failure to properly appreciate an effect. Given the large number of anti-anginal therapies that are ineffective in PAD it is perhaps not unsurprising that the outcome of this trial was negative. Nevertheless it was an important question to be answered given the small number of effective therapies that are on offer to these patients and in view of the multitude of other areas in which allopurinol has shown promise.

Given the findings presented here, one would question as to whether further research in this area would be worthwhile. A study involving a larger number of patients and either alternative vascular imaging techniques or stricter criteria around handrail use, shuttle walk testing and pre-FMD preparation may help to provide an absolutely definitive answer – however with no sign of any improvement despite good compliance with therapy it would perhaps not be the best use of research funds. Equally the use of alternative XO inhibitors such as febuxostat and oxypurinol would

not appear to be worthwhile given the levels of uric acid reduction demonstrated in this study. Allopurinol does of course bring other anti-oxidant effects and it may be that those could of some benefit in those patients who are earlier in the atherosclerotic disease process. However if one was looking earlier in the disease process this would need to be a longer-term project and thus one that would potentially be subject to confounding from changes in other variables over time. By comparison the promising results for allopurinol in patients with angina, due probably to it off-loading the LV, would suggest that angina is a more fruitful area for further research with this drug.

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Appendices

Examples of the trial paperwork are shown in the following pages.

All invitation letters, reply slips, study visit letters and post-trial notifications to patients were personalised to each participant and generated from the study database using the mail merge facility in Microsoft Word.

The CRF was printed as a double-sided booklet, with instructions/study visit progress noted on the facing page from the main visit content.

A. Participant invitation letter

UNIVERSITY OF DUNDEE

Medical Research Institute

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

Date: 05 July 2013

«GreetingLine»

I am a Clinical Research Fellow at the University of Dundee and am currently carrying out a study into a drug called allopurinol, to see if it is able to help patients with peripheral arterial disease to walk further before they experience leg pain.

As you attend the intermittent claudication/peripheral vascular disease clinic or have previously attended the vascular laboratory you are potentially eligible to participate in this study.

Please find enclosed a 'participant information sheet' which provides much more detail about the study and what taking part in it would involve – I would be very grateful if you would read through it and consider taking part.

If you are interested in taking part, or have any questions about the study then please fill out enclosed reply slip and return it to the Freepost address in the attached envelope.

Kind regards

Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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B. Participant information sheet

UNIVERSITY OF DUNDEE

Medical Research Institute

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

PARTICIPANT INFORMATION SHEET

Title of Study:
Allopurinol as a possible oxygen sparing agent in peripheral arterial disease

Name of Researcher:
Dr Alan J Robertson
(Chief Investigator: Professor Allan D Struthers)

Details of Study:
You are being invited to participate in a clinical trial at the Centre for Cardiovascular and Lung Biology, Ninewells Hospital and Medical School. Before you decide whether or not to take part it is important for you to understand why the research is being carried out and what it involved. Please take time to read the following information carefully and discuss it with others if you wish. Please ask if there is anything that is unclear or if you would like more information. Take your time to decide whether or not you would like to take part.

Background
Peripheral arterial disease refers to narrowing in the arteries supplying blood to the limbs. It is most commonly experienced by patients as a cramping pain in the legs that comes on whilst walking and rapidly settles when resting. Blood carries oxygen round the body and because of the narrowings in the arteries this means that that the leg muscles do not get as much oxygen as they need, causing the cramping pain. It has recently been found that a medication called allopurinol – a safe drug that has been used to treat gout for many years – has helped with another condition where oxygen demand outstrips oxygen supply, namely angina. Our theory is that this same medication may also help patients with peripheral arterial disease, by enabling them to walk further.

Do I have to take part?
Participation in this study is entirely voluntary and you are free to refuse to take part or withdraw from the study at any time (without having to give a reason) and without this in any way affecting your future medical care or your relationship with medical staff looking after you. Some insurance companies consider that participation in medical research such as this is a "material fact" which should be mentioned in any proposal for health-related insurance or which could influence their judgement in consideration of claims under existing policies. You should check that participation in this research does not affect any policy you might be thinking about taking out or any existing policy.

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t +44 (0)1382 383013 f +44 (0)1382 644972 e a.d.struthers@dundee.ac.uk

What is involved in the study?

The study takes six months to complete. It is a randomised, double-blind study. This means that you would either take a tablet which contains the medication we are testing (called allopurinol) or an inactive tablet (called a placebo). The tablet allocated to you is decided in a random way (a bit like tossing a coin) such that neither you nor the research staff will know which tablet you are taking until after the study is completed. This ensures that the study results cannot be influenced by knowing whether you are receiving the medication or not.

To make sure you are suitable for the study we first ask you to undertake two exercise treadmill tests. These will be done a week apart and are to ensure that your symptoms are stable. We'll also take some baseline blood tests and test the 'stiffness' of your arteries (full details below). We'll randomly allocate you to either the allopurinol treatment or the placebo and then follow you up regularly after starting treatment. Full details of the study visits are listed at the end of this information sheet, but for the whole trial there will be six hospital visits over six months and several telephone calls. With your agreement we would also like to store the blood samples we take for a period of five years so we can use it to test any new blood markers that become available in the near future.

Medication

The medication used in this study is called allopurinol. It was been around for about 50 years now although mainly for the treatment of gout. It has a good safety record and is generally well tolerated. However, like most medicines, allopurinol occasionally causes side effects. The most common side effect is nausea and some abdominal discomfort which affects less than one in ten of patients on allopurinol. This can be minimised by taking the tablets with food.

Allopurinol causes a skin rash in one in a hundred or less of patients. This may be associated with fever, swollen glands, joint pains, unusual blistering or bleeding. Were any of these symptoms to develop, you should stop taking the tablet immediately and contact the study doctor as soon as possible. You may also seek advice from your GP.

Reports of other side effects of allopurinol are very rare (less than 1 in 10,000 people) and it is not always clear if they are truly related to the treatment. The complete range of reported side effects is set out in a Patient Information Leaflet, a copy of which is attached for your information, but include headache, stomach upset, drowsiness, anaemia. This will be further discussed with you before you make a final decision about taking part in this study.

Placebo tablets are inactive tablets that just look the same as the active tablet but do not contain any of the allopurinol medication. We do not expect you to take any side effects are all whilst taking these. Your usual medication should be taken as normal.

Exercise test
This test involves you walking on a treadmill. We are trying to find out how far you can walk before you experience leg pain – the hope is that the treatment will improve this distance.

Six-minute walk test
This assesses your overall exercise ability. You will be asked to walk for six minutes along a level corridor at your own pace. You will be allowed to stop if you need to.

Questionnaires
We will use some questionnaires to ask you questions about your normal life and also your walking ability. Our hope is that we will be able to improve your walking ability (and perhaps your overall quality of life) with the active drug.

Assessing blood vessel 'stiffness'
At three of your visits we will measure blood vessel stiffness in your arm using non-invasive ultrasound. This technique is called "Flow Mediated Dilatation" (or FMD for short). To see how stiff the blood vessel is we need to see how it responds to having a short period where the circulation to the forearm is blocked and then to see how it responds to a drug called GTN (a drug that is also used to treat angina).

For this test you will be asked to fast for eight hours prior to the study (except for sips of water for taking your medication) and to hold off your morning nitrate tablet (if you are on such a medication) until later in the day after you have had the test. You will also be asked not to take exercise, smoke, consume caffeine, high fat food or vitamin C for the four hours leading up to the test as this would interfere with the results. You will be asked to lie on a couch for roughly half an hour. A blood pressure cuff will be placed below your elbow. We will then take an ultrasound measurement of the artery above the elbow (this involves placing an ultrasound probe and some jelly gently on the skin above the elbow). We will then inflate the blood pressure cuff to block the circulation to the forearm for a period of five minutes. You may find this a bit uncomfortable. After five minutes we will then release the cuff and take another scan to measure the artery above the elbow. We will then repeat this process this time before and after giving you a spray of GTN under the tongue.

Contraceptive Advice
Anyone who is pregnant cannot take part in this study. If you are a woman of childbearing age we will need to do a pregnancy test before the study. It is also important that you do not become pregnant during the study. Here is some advice on contraception. To avoid

getting pregnant, not having sex at all is obviously effective. If you follow this strictly, no contraception is needed. If not, these are effective types of contraception:

- Combined Oral Contraceptive Pill
- EVRA-osetrogen and progestogen: 'Transdermal Patch'
- Progestogen only pill: 'mini pill'
- Depoprovera injection (medroxyprogesterone acetate)
- Implanon Implant (Etonogestrel)
- Mirena Coil (Intra-Uterine System)
- IUD-copper containing intrauterine device
- Female sterilisation

Male vasectomy is also a good form of contraception but only if the procedure has been checked afterwards by your doctor to make sure it has worked.
No contraception method is 100% reliable by itself. Even surgical sterilisation in men and women has been known to fail very occasionally. We advise using additional contraception from the start of the study.

You may normally use 'barrier methods' such as the condom, diaphragm or cap. There is no definite proof that using a spermicide with a 'barrier method' gives extra protection but some condoms are manufactured with spermicide on them. If you require further advice on contraception, please ask.

What are the discomforts, risks and side effects?
The side effects of the allopurinol are discussed under the 'medication' section above.
Having blood tests taken can cause some mild bruising.
The GTN spray given for the FMD test can cause a slight headache although this usually passes quickly.
When the blood pressure cuff is inflated there can be some mild discomfort that goes away quickly once it is deflated.

What are the benefits of taking part in the study?
You will be monitored closely during the study and will be seen by a heart specialist at each of your study visits. Besides having tests that have already been mentioned, your medication will be reviewed on a regular basis. The tests will give us information about the function of your heart, kidneys and blood circulation. If any of these investigations reveal any new abnormality we will either discuss this with your GP or refer you to a specialist clinic at Ninewells Hospital (whichever seems most appropriate). The study may not immediately benefit you, but if the results of the study are positive this may change the practice of managing patients with peripheral arterial disease like you and potentially will have a great impact on thousands or even millions of patients in the future. If so, you may gain eventually from our discovering a new treatment for your condition.

What are my rights?
If you wish the results of the study can be made available to you or your GP when the study is complete.

If you have a complaint about your participation in the study you should first talk to the Investigator involved in your care. You can ask to speak to a senior member of the Centre for Cardiovascular and Lung Biology or the Complaints Officer for NHS Tayside.

Complaints and Claims Manager
Complaints and Advice Team
Level 7, Ninewells Hospital
Dundee DD1 9SY
Freephone: 0800 027 5507
Email: nhstaysidecomplaints@thb.scot.nhs.uk

In the event that something goes wrong and you are harmed during the study there are no special compensation arrangements. If you are harmed and this is due to someone's negligence then you may have grounds for a legal action for compensation against the University of Dundee or NHS Tayside but you may have to pay your legal costs. The normal National Health Service complaints mechanisms will still be available to you (if appropriate).

Will the research influence the treatment I receive?
The research will not alter the regular treatments you receive.

Will my taking part in the study be kept confidential?
With your permission, identifiable information about you and data collected during the study will be held by the Institute of Cardiovascular Research, Ninewells Hospital and Medical School. All data collected in this study will be coded and stored on a computer system protected by a password only available to the researchers. No one outside the research team will have access to any identifiable information and all identifiable information and data will be kept securely. With your permission, we will inform your GP of your participation in this study. It is a requirement of the regulators that your records in this study, together with any other relevant medical records, be made available for scrutiny by appropriate staff from NHS Tayside, University of Dundee (or their appointed third party) and the regulatory authorities.

Will I continue to receive the medication used in this study after it finishes?
There is no guarantee of this. The study is designed to give an indication of possible benefit from the medicine being tested and it may be some time before we can be sure about how useful it actually is.

Who has reviewed this study?
The Scotland A Research Ethics Committee, which has the authority to scrutinise proposals for medical research on humans in Scotland, has examined this study and has raised no objections from the point of view of medical research.

If you are worried at any time about the research or wish to discuss things generally further, please do not hesitate to contact:

Dr Alan J Robertson MBChB, MRCP (UK)
BHF Clinical Research Fellow
Centre for Cardiovascular & Lung Biology
Division of Medical Sciences
Mail Box 2, Ninewells Hospital and Medical School
Dundee DD1 9SY
Tel: (01382) 632180
Email: a.s.robertson@dundee.ac.uk

What will happen to me during the study?
The following is the programme of visits involved in this study - we will provide a taxi or travel expenses for all of the visits.

- Visit 1 (week 0) – screening visit 1
    - Consent – answer any outstanding questions you may have and complete the consent form.
    - Measurement of blood pressure in arms and legs
    - Treadmill test
    - Blood samples

- Visit 2 (week 0) – screening visit 2
    - Treadmill test – if this is stable and similar to the previous test then you are able to continue in the study
    - Six minute walk test
    - Measurement of blood vessel 'stiffness'
    - Supply of initial study medication along with instructions.
    - Two questionnaires - Walking Impairment and Quality of Life

- Visit 3 (week 6) – progress visit
    - Check how you are doing on the medications
    - Blood samples

- Supply of study medication for the remainder of the study

- Visit 4 (week 12) – progress visit
  - Treadmill test
  - Six minute walk test
  - Check how you are doing on the medications
  - Two questionnaires - Walking Impairment and Quality of Life

- Visit 5 (week 18) – progress visit
  - Measurement of blood vessel 'stiffness'
  - Check how you are doing on the medications
  - Blood samples

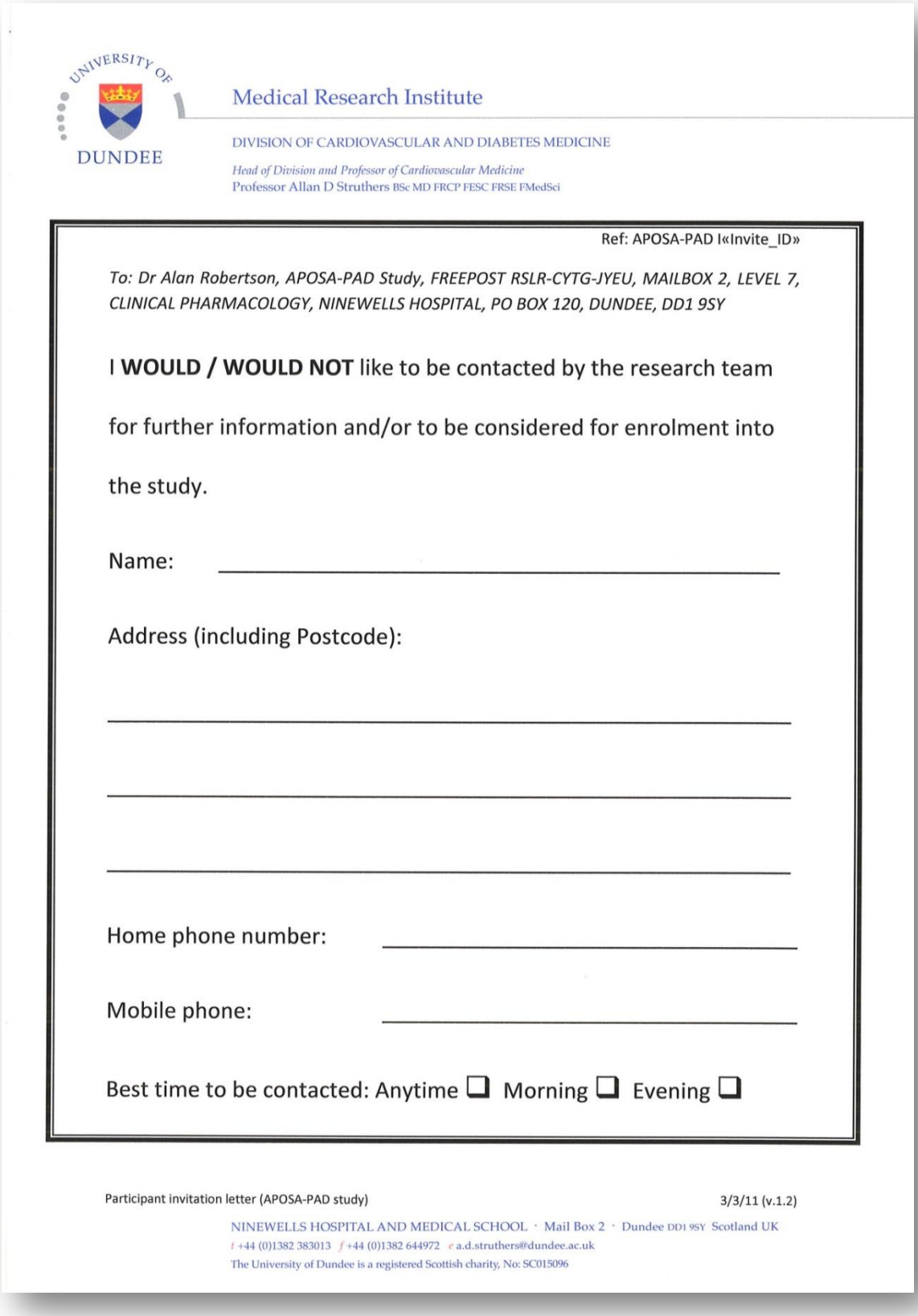
- Visit 6 (week 24) – final visit
  - Measurement of blood vessel 'stiffness'
  - Treadmill test
  - Six minute walk test
  - Measurement of blood pressure in arms and legs
  - Check how you are doing on the medications
  - Blood samples
  - Two questionnaires - Walking Impairment and Quality of Life

Contact Numbers

If during the study you become unwell or are concerned, as well as the usual services provided by the NHS such as NHS24 (08454 242424), you can also contact the study team during normal working hours on (01382) 632180. If you are unwell and need urgent advice or assistance do not delay in seeking further advice or treatment as usual through the NHS services.

Thank you for reading this information sheet and considering taking part in this study. If you would like more information or want to ask questions about the study please contact the study team on the number above.

C. Participant reply slip



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DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

Ref: APOSA-PAD I«Invite_ID»

To: Dr Alan Robertson, APOSA-PAD Study, FREEPOST RSLR-CYTG-JYEU, MAILBOX 2, LEVEL 7, CLINICAL PHARMACOLOGY, NINEWELLS HOSPITAL, PO BOX 120, DUNDEE, DD1 9SY

I **WOULD / WOULD NOT** like to be contacted by the research team for further information and/or to be considered for enrolment into the study.

Name: _____

Address (including Postcode):

_____

_____

_____

Home phone number: _____

Mobile phone: _____

Best time to be contacted: Anytime ❑ Morning ❑ Evening ❑

Participant invitation letter (APOSA-PAD study) 3/3/11 (v.1.2)

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The University of Dundee is a registered Scottish charity, No: SC015096

D. Participant consent form

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Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

Study Number: 2010-020662-23 (EudraCT)
Participant Identification Number for this trial: .....

PARTICIPANT CONSENT FORM

Title of Study:
Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Name of Researcher:
Dr Alan J Robertson
(Chief Investigator: Professor Allan D Struthers)

Please initial box

1. I confirm that I have read and understand the information sheet dated ..... (ver .....) for the above study. I have had the opportunity to consider the information, to ask questions, and have had them answered satisfactorily. ☐
2. I understand that my participation is voluntary and that I am free to withdraw at any time, without giving any reason, without my medical care or legal rights being affected. ☐
3. I understand that relevant sections of my medical notes and data collected during the study may be looked at by the research team or from the regulatory authorities, NHS Tayside, or the University of Dundee (or their appointed third party), where it is relevant to my taking part in this study. I give permission for these individuals to have access to my records. ☐
4. I agree to my GP being informed of my participation in this study. ☐
5. I agree to take part in the above study. ☐

Name of participant	Date	Signature
Name of person taking consent	Date	Signature

1 copy for participant; 1 copy for trial master file; 1 original to be kept with hospital notes

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E. Visit confirmation letters

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Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

5 July 2013

01382 632180

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Many thanks once again for agreeing to consider participating in this study.

As discussed on the telephone, I look forward to seeing you at the Department of Clinical Pharmacology at the following time:

«Visit_1»

[insert taxi details if relevant]

Please find enclosed directions of how to find the department/arrange to be picked up from reception.

Please bring a list of your current medication with you.

Kind regards

Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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5 July 2013

01382 632180

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Many thanks for your ongoing participation in this study. I look forward to seeing you at the Department of Clinical Pharmacology at the following time for your next visit:

«Visit_2»
[insert taxi details if relevant]

At this visit we will be repeating the treadmill test and also doing a short walking test in the corridor, a test of blood vessel 'stiffness' and a couple of questionnaires.

The blood vessel 'stiffness' test has a couple of special requirements: <u>in the four hours before attending</u>, please:

- **don't have anything significant to eat (light breakfast/lunch is OK)**
- **don't have any tea/coffee (water/juice is OK)**
- **don't have any cigarettes**

Kind regards
Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

PS – please find enclosed a copy of the consent form that you signed at the last visit.

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5 July 2013

01382 632180

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Many thanks for your ongoing participation in this study. I look forward to seeing you at the Department of Clinical Pharmacology at the following time for your next visit:

«Visit_3»
[insert taxi details if relevant]

At this visit we will be taking some blood samples and providing the next supply of study medication.

Please bring all study medication & bottles with you, along with a note of your current medications.

Kind regards
Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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5 July 2013

01382 632180

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Many thanks for your ongoing participation in this study. I look forward to seeing you at the Department of Clinical Pharmacology at the following time for your next visit:

«Visit_4»
[insert taxi details if relevant]

At this visit we will be repeating the treadmill test, the short walking test in the corridor and the questionnaires. We will also provide a further supply of study medication.

Please bring all study medication & bottles with you, along with a note of your current medications.

Kind regards
Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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5 July 2013

01382 632180

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Many thanks for your ongoing participation in this study. I look forward to seeing you at the Department of Clinical Pharmacology at the following time for your next visit:

«Visit_5»
[insert taxi details if relevant]

At this visit we will be repeating the blood vessel 'stiffness' test and taking some blood samples.

As you will recall, the blood vessel 'stiffness' test has a couple of special requirements: <u>in the four hours before attending</u>, please:

- **don't have anything significant to eat (light breakfast/lunch is OK)**
- **don't have any tea/coffee (water/juice is OK)**
- **don't have any cigarettes**

<u>Please bring all study medication & bottles with you</u>, along with a note of your current medications.

Kind regards
Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

5 July 2013

01382 632180

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Many thanks for your ongoing participation in this study. I look forward to seeing you at the Department of Clinical Pharmacology at the following time for your final study visit:

«Visit_6»
[insert taxi details if relevant]

At this visit we will be repeating the treadmill test, the short walking test in the corridor, the questionnaire, the blood vessel 'stiffness' test and taking some blood samples.

As you will recall, the blood vessel 'stiffness' test has a couple of special requirements: <u>in the four hours before attending</u>, please:

- **don't have anything significant to eat (light breakfast/lunch is OK)**
- **don't have any tea/coffee (water/juice is OK)**
- **don't have any cigarettes**

<u>Please bring all study medication & bottles with you</u>, along with a note of your current medications.

Kind regards
Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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F. IMP instruction sheet

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Medical Research Institute

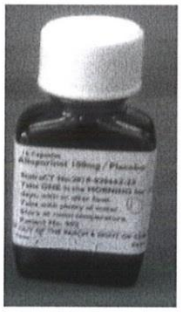
Tel: 01382 740 102

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

APOSA-PAD STUDY MEDICATION INSTRUCTIONS

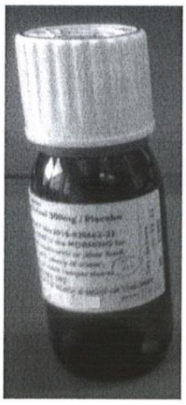
For the first six weeks of the study, the medication is supplied in two bottles:



Weeks 1 and 2

One tablet per day, in the morning

(smaller, square bottle)



Weeks 3 - 6

One tablet per day, in the morning

(larger, rounded bottle)

<u>Please note</u> there are a couple of tablets spare in both bottles, therefore after completing two weeks with bottle one you will still have a couple left – don't worry about that, just move on to bottle two.

Please remember to bring all bottles and leftover tablets up on your study visits.

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G. Letter to participant's GP

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DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

Date: _____

Dear Colleague

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

This letter is to inform you that your patient _____ (CHI: _____) has kindly agreed to participate in the above research study at the Centre for Cardiovascular and Lung Biology, Ninewells Hospital and Medical School, Dundee.

It is a randomised, double-blinded, placebo-controlled study assessing whether allopurinol improves the distance walked in patients with peripheral arterial disease. The main endpoint will be an exercise tolerance test, supplemented by quality of life questionnaires.

High dose allopurinol has recently been shown to have strong anti-ischaemic effects in angina pectoris, where it prolongs the time to ST depression during exercise by as much as 25%. So far, this anti-ischaemic effect of allopurinol has only been demonstrated in cardiac tissue and the key question now is whether this "oxygen sparing" or anti-ischaemic effect of allopurinol occurs also in non-cardiac ischaemic tissues. Peripheral arterial disease is a particularly relevant disease in which to test this idea since coincidental coronary disease is very common in PAD and hence an anti-ischaemic effect in both the heart and the legs would be particularly welcome.

The study takes six months to complete, during which time the patient will be reviewed regularly and as well as undertaking exercise tests they will also have regular monitoring of their FBC, UEs and LFTs.

One potential interaction I would like to draw your attention to is that there has been reported to be an increase in frequency of skin rash among patients receiving ampicillin or amoxicillin concurrently with allopurinol compared to patients who are not receiving both drugs. The cause of the reported association has not been established. However, it is recommended (in the Summary of Product Characteristics) that "in patients receiving allopurinol an alternative to ampicillin or amoxicillin is used where available".

The results will be analysed at the end of the overall two year study period and a copy of this analysis will be forwarded to you. Please do not hesitate to get in touch with me if you require any further information about the study. I enclose a Participant Information Sheet for your information.

Kind regards

Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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H. Walking Impairment Questionnaire

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Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

APOSA-PAD Participant ID:

.....

WALKING IMPAIRMENT QUESTIONNAIRE

This survey aims to assess how far you are able to walk on a day-to-day basis.

For each of the following questions, please select the one response that best describes your answer.

1. Please tick the box that best describes how much difficulty you have had walking due to pain, aches or cramps during the last week.

During the last week, how much difficulty have you had walking due to:	No difficulty	Slight difficulty	Some difficulty	Much difficulty	Great difficulty
a. Pain, aching, or cramps in your calves?	❑	❑	❑	❑	❑
b. Pain, aching, or cramps in your buttocks?	❑	❑	❑	❑	❑

For the following questions we are trying to gauge how far you can walk before you have to stop due to leg pain or discomfort. If you would not manage to do the activity without getting pain/discomfort then please tick "**Unable to do**".

However if you do not perform the activity for other reasons (such as you do not have stairs at home) then please tick "**Didn't do for other reasons**".

2. Please tick the box that best describes how hard it was for you to walk on level ground without stopping for each of the following distances during the last week:

During the last week, how difficult was it for you to:	No difficulty	Slight difficulty	Some difficulty	Much difficulty	Unable to do	Didn't do for other reasons
a. Walk indoors, such as around your home?	❑	❑	❑	❑	❑	❑
b. Walk 50 feet?	❑	❑	❑	❑	❑	❑
c. Walk 150 feet?	❑	❑	❑	❑	❑	❑
d. Walk 300 feet (100 yards)?	❑	❑	❑	❑	❑	❑
e. Walk 600 feet (200 yards)?	❑	❑	❑	❑	❑	❑
f. Walk 900 feet (300 yards)?	❑	❑	❑	❑	❑	❑
g. Walk 1500 feet (~¼ mile)?	❑	❑	❑	❑	❑	❑

v.1.1 (7/7/10) Source: Regensteiner JG, Steiner JF, Panzer RJ. Evaluation of walking impairment by questionnaire in patients with peripheral arterial disease. *J Vasc Med Biol* 1990;2:142-52.

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3. Please tick the box that best describes how hard it was for you to walk one city block on level ground at each of these speeds without stopping to rest during the last week.

During the last week, how difficult was it for you to:	No difficulty	Slight difficulty	Some difficulty	Much difficulty	Unable to do	Didn't do for other reasons
a. Walk 300ft (100 yards) slowly?	❑	❑	❑	❑	❑	❑
b. Walk 300ft (100 yards) at average speed?	❑	❑	❑	❑	❑	❑
c. Walk 300ft (100 yards) quickly?	❑	❑	❑	❑	❑	❑
d. Run or jog 300ft (100 yards)?	❑	❑	❑	❑	❑	❑

4. Please tick the box that best describes how hard it was for you to climb stairs without stopping to rest during the past week. Please note one flight of stairs is roughly equal to 14 steps.

During the last week, how difficult was it for you to:	No difficulty	Slight difficulty	Some difficulty	Much difficulty	Unable to do	Didn't do for other reasons
a. Climb 1 flight of stairs?	❑	❑	❑	❑	❑	❑
a. Climb 2 flights of stairs?	❑	❑	❑	❑	❑	❑
a. Climb 3 flights of stairs?	❑	❑	❑	❑	❑	❑

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

v.1.1 (7/7/10) Source: Regensteiner JG, Steiner JF, Panzer RJ. Evaluation of walking impairment by questionnaire in patients with peripheral arterial disease. *J Vasc Med Biol* 1990;2:142-52.

I. SF-36 Questionnaire

UNIVERSITY OF DUNDEE

Medical Research Institute

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

APOSA-PAD Participant ID:

.....

QUALITY OF LIFE QUESTIONNAIRE

This survey asks for your views about your health. This information will help keep track of how you feel and how well you are able to do your usual activities.

For each of the following questions, please select the one response that best describes your answer.

1. In general, would you say your health is:

Excellent	Very good	Good	Fair	Poor
❑	❑	❑	❑	❑

2. Compared to one year ago, how would you rate your health in general now?

Much better now than one year ago	Somewhat better now than one year ago	About the same as one year ago	Somewhat worse now than one year ago	Much worse now than one year ago
❑	❑	❑	❑	❑

3. The following questions are about activities you might do during a typical day. Does your health now limit you in these activities? If so, how much?

	Yes, a lot	Yes, a little	No, not at all
a. Vigorous activities, such as running, lifting heavy objects, participating in strenuous sports	❑	❑	❑
b. Moderate activities, such as moving a table, pushing a vacuum cleaner, bowling, or playing golf	❑	❑	❑
c. Lifting or carrying groceries	❑	❑	❑
d. Climbing several flights of stairs	❑	❑	❑
e. Climbing one flight of stairs	❑	❑	❑
f. Bending, kneeling, or stooping	❑	❑	❑
g. Walking more than a mile	❑	❑	❑
h. Walking several hundred yards	❑	❑	❑
i. Walking one hundred yards	❑	❑	❑
j. Bathing or dressing yourself	❑	❑	❑

v.1.0 (31/5/10) SF-36® is a registered trademark of the Medical Outcomes Trust

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4. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of your physical health?

		Yes	No
a.	Cut down on the amount of time you spent on work or other activities	❑	❑
b.	Accomplished less than you would like	❑	❑
c.	Were limited in the kind of work or other activities	❑	❑
d.	Had difficulty performing the work or other activities (for example, it took extra effort)	❑	❑

5. During the past 4 weeks, have you had any of the following problems with your work or other regular daily activities as a result of any emotional problems (such as feeling depressed or anxious)?

		Yes	No
a.	Cut down on the amount of time you spent on work or other activities	❑	❑
b.	Accomplished less than you would like	❑	❑
c.	Did work or activities less carefully than usual	❑	❑

6. During the past 4 weeks, to what extent has your physical health or emotional problems interfered with your normal social activities with family, friends, neighbours, or groups?

Not at all	Slightly	Moderately	Quite a bit	Extremely
❑	❑	❑	❑	❑

7. How much bodily pain have you had during the past 4 weeks?

None	Very mild	Mild	Moderate	Severe	Very severe
❑	❑	❑	❑	❑	❑

8. During the past 4 weeks, how much did pain interfere with your normal work (including both work outside the home and housework)?

Not at all	Slightly	Moderately	Quite a bit	Extremely
❑	❑	❑	❑	❑

9. These questions are about how you feel and how things have been with you during the past 4 weeks. For each question, please give the one answer that comes closest to the way you have been feeling. How much of the time during the past 4 weeks.

	All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
a. Did you feel full of life?	❑	❑	❑	❑	❑	❑
b. Have you been very nervous?	❑	❑	❑	❑	❑	❑
c. Have you felt so down in the dumps that nothing could cheer you up?	❑	❑	❑	❑	❑	❑
d. Have you felt calm and peaceful?	❑	❑	❑	❑	❑	❑
e. Did you have a lot of energy?	❑	❑	❑	❑	❑	❑
f. Have you felt downhearted and depressed?	❑	❑	❑	❑	❑	❑
g. Did you feel worn out?	❑	❑	❑	❑	❑	❑
h. Have you been a happy person?	❑	❑	❑	❑	❑	❑
i. Did you feel tired?	❑	❑	❑	❑	❑	❑

10. During the past 4 weeks, how much of the time has your physical health or emotional problems interfered with your social activities (like visiting friends, relatives, etc.)?

All of the time	Most of the time	A good bit of the time	Some of the time	A little of the time	None of the time
❑	❑	❑	❑	❑	❑

11. How TRUE or FALSE is each of the following statements for you?

	Definitely true	Mostly true	Don't know	Mostly false	Definitely false
a. I seem to get sick a little easier than other people	❑	❑	❑	❑	❑
b. I am as healthy as anybody I know	❑	❑	❑	❑	❑
c. I expect my health to get worse	❑	❑	❑	❑	❑
d. My health is excellent	❑	❑	❑	❑	❑

THANK YOU FOR COMPLETING THIS QUESTIONNAIRE

J. CRF (including FMD protocol)

NHS Tayside

The APOSA-PAD Study

UNIVERSITY OF DUNDEE

CASE REPORT FORM

A randomised, placebo-controlled trial of allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease.

Participant Initials	
Screening #	
Randomisation #	
CRF Start Date	

Visit	Date	Taxi Required?	Taxi Booked?	Pre-visit reminder phone call	Comment
1 (week 0) Screening 1					
2 (week 0) Screening 2					
3 (week 6) Progress					
4 (week 12) Progress					
5 (week 18) Progress					
6 (week 24) Final					
Extra					
Extra					

Study visits overview

- Visit 1 (week 0) – screening visit 1
  - Participant consent – answer any outstanding questions and complete consent form.
  - Baseline ABI
  - Baseline ETT
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Record list of current medications

- Visit 2 (week 0) – screening visit 2
  - Second baseline ETT – if stable (<25% variance) then can continue in study
  - Six minute walk test
  - FMD measurement
  - Supply of initial study medication to participant along with instructions.
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

- Visit 3 (week 6) – progress visit
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/isoprostanes/oxidised LDL
  - Record list of current medications
  - Supply of full dose study medication (first half)

- Visit 4 (week 12) – progress visit
  - ETT
  - Six minute walk test
  - Assess medication compliance.
  - Check for AEs
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications
  - Supply of full dose study medication (second half)

- Visit 5 (week 18) – progress visit
  - FMD measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT
  - Record list of current medications

- Visit 6 (week 24) – final visit
  - FMD measurement
  - ETT
  - Six minute walk test
  - ABI measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

APOSA-PAD Subject ID S.....R..... Initials ..... Date: __/__/_____ Visit 1

Visit 1 (Screening visit 1 of 2)

Participant consent – answer any outstanding questions and complete consent form.

V1A	**Has a consent form been completed & filed in the TMF?**	

Check inclusion/exclusion criteria.

Inclusion

1. Is the subject aged between 35-85 years?	Yes / No
2. Does the subject suffer from peripheral arterial disease, which is defined as:	
• 2.1 leg pain on walking and disappearing within 10 minutes on standing and of presumed atherosclerotic origin?	Yes / No
• 2.2 an ankle brachial pressure index (ABI) of <0.90 on the worst leg at rest?	Yes / No

V1B	**Does the subject meet inclusion criteria?**	

Exclusion

1. Does the subject suffer from rest pain?	Yes / No
2. Is the subject pregnant, breast-feeding or a woman of child-bearing potential not using adequate contraception?	Yes / No
3. Does the subject suffer from heart failure or other exercise limiting cardiac disease?	Yes / No
4. Is the subject's BP known to be >180mmHg (systolic) or >100mmHg (diastolic)?	Yes / No
5. Is the subject known to suffer from malignancy, chronic kidney or liver disease?	Yes / No
6. Is the subject already on allopurinol or known to have an adverse reaction to it?	Yes / No
7. Has the subject participated in another clinical trial (other than observational trials and registries) concurrently or within 30 days prior to screening for entry into this study?	Yes / No
8. Does the subject have other serious illness or significant abnormalities that may compromise their safety or successful participation in the study?	Yes / No
9. Does the subject have any illness which in the doctor's opinion means that the subject is unable to give informed consent?	Yes / No
10. Has the subject had any cardiovascular disease event within the last three months like MI, unstable angina, stroke?	Yes / No
11. Has the subject had a recent marked change in symptoms or had intervention for PAD in the last six months?	Yes / No
12. Is the subject receiving treatment with either 6-mercaptopurine, azathioprine, warfarin, or theophylline?	Yes / No

V1C	**Is the subject free of any exclusion criteria at this stage?**	

Unless the answers to questions V1A-V1C are all YES then the subject may not continue any further in the study.

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Visit 1 (continued)

History (NB – list medications in the Medications Log on page 18 of the CRF)

Past Medical History

Family History

Social History

Smoking status:

❑ current smoker ❑ non-smoker ❑ previous smoker

cigarettes/day (A) _____ # years smoked (B) _____ pack years _____ (AxB/20)

Average weekly alcohol intake:

Occupation:

Home circumstances:

Other:

Examination

General	Age _____	Height _____ m		BP ____/____ mmHg
		Weight _____ kg		Pulse _____ bpm
		BMI _____ kg/m^2		Male / Female
Cardiovascular			*Respiratory*	
Gastrointestinal			*Neurological*	

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V1D	Medical history (incl. medication list) and physical examination complete	

Venepuncture

Samples required:

- 1x EDTA (purple 4ml) – FBC
- 1x EDTA (pink 10ml) – isoprostanes
- 1x EDTA (pink 5ml) – oxidised LDL
- 2x clotted (yellow 5ml) – UE/LFT/uric acid, storage

V1E	Blood samples taken for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL/storage	

Baseline ABI

Remember: ABI= highest ankle pressure for each side ÷ highest Brachial BP

	Right	*Left*
Brachial BP		
Posterior tibial BP		
Dorsalis pedis BP		
ABI		

Instructions for selecting Gardner Protocol:

1. Turn on treadmill at back and side
2. Login to system (Username: User; Password: User)
3. Select> NEW TEST
4. Create> NEW PATIENT and Enter: Patient ID (APOSA-PAD xxx); Gender and DOB
5. Click> ACCEPT and >SELECT
6. Go to Test Info and Select> GARDNER then >OK
7. Press> PRE-TEST (Blue Button)
8. With participant straddling the treadmill, press> START TREADMILL (Button)
9. Walk for 5-10 sec max at each speed. When Pre-test completed Press> STOP TREADMILL (Red Button) and instruct participant to wait until treadmill has stopped
10. Repeat steps 7-9 for speeds of 1, 1.5 and 2mph
11. Attach ECG electrodes and connect leads to participant during the 10 minute rest period
12. With participant straddling the treadmill, press> PRE-TEST and > START TREADMILL
13. When treadmill up to speed, participant steps on
14. When second heel hits the treadmill press> EXERCISE (Button)
15. When the participant reports onset of claudication symptoms, Press> 12 LEAD (Button) (this will print an ECG). Also note when treadmill speed reaches 2mph
16. An ECG will be printed 10 seconds prior to each change in gradient
17. When participant reports Absolute Claudication press> 12 LEAD (Button) then >RECOVERY or >TEST END. Make sure to always press> 12 LEAD before stopping test.
18. Print only CONFIGURED TEST
19. Do not save ECG data

Baseline ETT

Follow instructions as per protocol on opposite page.

Claudication onset @ ___min : ___sec
Peak walking time (PWT) @ ___min : ___sec
Grade at PWT: ____

Primary reason for stopping the treadmill:

❑ Claudication

❑ Other, specify _____

Leg which was affected most during the test: ❑ Right ❑ Left

Description of claudication symptoms:

Right _____ (e.g., calf)	❑ Fatigue ❑ Cramp ❑ Pain ❑ Other, describe _____
Left _____ (e.g., calf)	❑ Fatigue ❑ Cramp ❑ Pain ❑ Other, describe _____

V1F	**ABIs recorded; ETT carried out and results printed & filed in CRF**	

Final Visit 1 checks

V1G	**Date for second screening visit booked and recorded on front of CRF**	

Signed		**Name**		**Date**	

Study visits overview

- Visit 1 (week 0) – screening visit 1
  - Participant consent – answer any outstanding questions and complete consent form.
  - Baseline ABI
  - Baseline ETT
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Record list of current medications

- **Visit 2 (week 0) – screening visit 2**
  - **Second baseline ETT – if stable (<25% variance) then can continue in study**
  - **Six minute walk test**
  - **FMD measurement**
  - **Supply of initial study medication to participant along with instructions.**
  - **Walking Impairment Questionnaire**
  - **Quality of life (SF-36 questionnaire)**
  - **Record list of current medications**

- Visit 3 (week 6) – progress visit
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/isoprostanes/oxidised LDL
  - Record list of current medications
  - Supply of full dose study medication (first half)

- Visit 4 (week 12) – progress visit
  - ETT
  - Six minute walk test
  - Assess medication compliance.
  - Check for AEs
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications
  - Supply of full dose study medication (second half)

- Visit 5 (week 18) – progress visit
  - FMD measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT
  - Record list of current medications

- Visit 6 (week 24) – final visit
  - FMD measurement
  - ETT
  - Six minute walk test
  - ABI measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

Pre Visit 2 phone-call

- Confirm patient appointment time/date/transport
- Remind them of FMD requirements:
  - not eating for four hours before attending
  - no tea/coffee for four hours before attending
  - no cigarettes for four hours before attending

V2Pre	**Complete reminder phone-call checkbox on front of CRF**	

Visit 2

V2A	**Visit 1 blood results documented**	
V2B	**Medications log updated**	
V2C	**Adverse event log updated**	

Instructions for selecting Gardner Protocol:

1. Turn on treadmill at back and side
2. Login to system (Username: User; Password: User)
3. Select> NEW TEST
4. Create> NEW PATIENT and Enter: Patient ID (APOSA-PAD xxx); Gender and DOB
5. Click> ACCEPT and >SELECT
6. Go to Test Info and Select> GARDNER then >OK
7. Press> PRE-TEST (Blue Button)
8. With participant straddling the treadmill, press> START TREADMILL (Button)
9. Walk for 5-10 sec max at each speed. When Pre-test completed Press> STOP TREADMILL (Red Button) and instruct participant to wait until treadmill has stopped
10. Repeat steps 7-9 for speeds of 1, 1.5 and 2mph
11. Attach ECG electrodes and connect leads to participant during the 10 minute rest period
12. With participant straddling the treadmill, press> PRE-TEST and > START TREADMILL
13. When treadmill up to speed, participant steps on
14. When second heel hits the treadmill press> EXERCISE (Button)
15. When the participant reports onset of claudication symptoms, Press> 12 LEAD (Button) (this will print an ECG). Also note when treadmill speed reaches 2mph
16. An ECG will be printed 10 seconds prior to each change in gradient
17. When participant reports Absolute Claudication press> 12 LEAD (Button) then >RECOVERY or >TEST END. Make sure to always press> 12 LEAD before stopping test.
18. Print only CONFIGURED TEST
19. Do not save ECG data

Second baseline ETT

Follow instructions as per protocol on opposite page.

Claudication onset @ ___min : ___sec

Peak walking time (PWT) @ ___min : ___sec

Grade at PWT: ____

Primary reason for stopping the treadmill:

❑ Claudication

❑ Other, specify _____

Leg which was affected most during the test: ❑ Right ❑ Left

Description of claudication symptoms:

Right _____ (e.g., calf)	❑ Fatigue ❑ Cramp ❑ Pain ❑ Other, describe _____
Left _____ (e.g., calf)	❑ Fatigue ❑ Cramp ❑ Pain ❑ Other, describe _____

To participate in this study the subject will have to demonstrate stable disease by having a reproducible pain free walking distance (i.e. <25% variance) on two consecutive treadmill tests. The reason for termination of the test must be claudication pain only.

V2D	**Less than 25% variance with first baseline ETT confirmed (if NOT then explain to participant why they are unable to progress further in study)**	

Whilst the participant is resting from the ETT and prior to 6 minute walk test being carried out the two study questionnaires should be completed:

V2E	**Administer Walking Impairment questionnaire**	
V2F	**Administer SF-36 Quality of Life questionnaire**	
V2G	**FMD measurements carried out**	

Study week	Dose	Bottle contents	Bottle week
0	Nil	Nil	Nil
1	100mg od	Bottle 1 (16x100mg w/2 caps overage)	1
2	100mg od		2
3	300mg od	Bottle 2 (30x300mg w/2 caps overage)	1
4	300mg od		2
5	300mg od		3
6	300mg od		4
7	300mg bd	Bottle 3 (135x300mg w/9 caps overage)	1
8	300mg bd		2
9	300mg bd		3
10	300mg bd		4
11	300mg bd		5
12	300mg bd		6
13	300mg bd		7
14	300mg bd		8
15	300mg bd		9
16	300mg bd	Bottle 4 (135x300mg w/9 caps overage)	1
17	300mg bd		2
18	300mg bd		3
19	300mg bd		4
20	300mg bd		5
21	300mg bd		6
22	300mg bd		7
23	300mg bd		8
24	300mg bd		9

Six Minute Walk Test

Ten minute rest period pre-test ❑

Pre-test resting HR _____ bpm Under 120bpm? ❑

Pre-test resting BP _____ /_____ mmHg Under 180mmHg systolic & 100mmHg diastolic? ❑

Pre-test fatigue level on Borg scale _____

Number of 30m laps completed _____ = ____ x 30m = _____m total distance

Post-test fatigue level on Borg scale _____

If stopped prematurely

Time walked ____min ____ sec

Reason for early end of test: _____

_____

V2H	**Six minute walk test completed**	

Study medication

Number of tablets supplied _____

Randomisation number _____ (ensure this is then added to all CRF pages)

V2I	**Bottles 1 & 2 of study medication issued, complete with instructions**	

Final Visit 2 checks

V2J	**Letter to GP re. participation completed and sent out**	
V2K	**Copy of letter to GP filed in case notes & sticker placed on case notes**	
V2L	**Date for third visit booked and recorded on front of CRF**	

Signed		**Name**		**Date**	

Study visits overview

- Visit 1 (week 0) – screening visit 1
  - Participant consent – answer any outstanding questions and complete consent form.
  - Baseline ABI
  - Baseline ETT
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Record list of current medications

- Visit 2 (week 0) – screening visit 2
  - Second baseline ETT – if stable (<25% variance) then can continue in study
  - Six minute walk test
  - FMD measurement
  - Supply of initial study medication to participant along with instructions.
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

- **Visit 3 (week 6) – progress visit**
  - **Assess medication compliance.**
  - **Check for AEs**
  - **Bloods for FBC/UE/LFT/isoprostanes/oxidised LDL**
  - **Record list of current medications**
  - **Supply of full dose study medication (first half)**

- Visit 4 (week 12) – progress visit
  - ETT
  - Six minute walk test
  - Assess medication compliance.
  - Check for AEs
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications
  - Supply of full dose study medication (second half)

- Visit 5 (week 18) – progress visit
  - FMD measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT
  - Record list of current medications

- Visit 6 (week 24) – final visit
  - FMD measurement
  - ETT
  - Six minute walk test
  - ABI measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

APOSA-PAD Subject ID S.....R..... Initials ..... Date: __/__/_____ Visit 3

Pre Visit 3 phone-call

- Confirm patient appointment time/date/transport

V3Pre	**Complete reminder phone-call checkbox on front of CRF**	

Visit 3

V3A	**Medications log updated**	
V3B	**Adverse event log updated**	

Venepuncture

Samples required:

- 1x EDTA (purple 4ml) – FBC
- 1x EDTA (pink 10ml) – isoprostanes
- 1x EDTA (pink 5ml) – oxidised LDL
- 2x clotted (yellow 5ml) – UE/LFT, storage

V3C	**Blood samples taken for FBC/UE/LFT/isoprostanes/oxidised LDL/storage**	

Medication compliance

Number of tablets remaining _____

Comments _____

_____

_____

V3D	**Medication compliance checked**	

Study medication supply

Number of tablets supplied _____

V3E	**Bottle 3 of study medication issued, complete with instructions**	

Final Visit 3 checks

V3F	**Date for fourth visit booked and recorded on front of CRF**	

Signed		**Name**		**Date**	

Study visits overview

- Visit 1 (week 0) – screening visit 1
  - Participant consent – answer any outstanding questions and complete consent form.
  - Baseline ABI
  - Baseline ETT
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Record list of current medications

- Visit 2 (week 0) – screening visit 2
  - Second baseline ETT – if stable (<25% variance) then can continue in study
  - Six minute walk test
  - FMD measurement
  - Supply of initial study medication to participant along with instructions.
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

- Visit 3 (week 6) – progress visit
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/isoprostanes/oxidised LDL
  - Record list of current medications
  - Supply of full dose study medication (first half)

- **Visit 4 (week 12) – progress visit**
  - **ETT**
  - **Six minute walk test**
  - **Assess medication compliance.**
  - **Check for AEs**
  - **Walking Impairment Questionnaire**
  - **Quality of life (SF-36 questionnaire)**
  - **Record list of current medications**
  - **Supply of full dose study medication (second half)**

- Visit 5 (week 18) – progress visit
  - FMD measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT
  - Record list of current medications

- Visit 6 (week 24) – final visit
  - FMD measurement
  - ETT
  - Six minute walk test
  - ABI measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

APOSA-PAD Subject ID S.....R..... Initials ..... Date: __ /__ /_____ Visit 4

Pre Visit 4 phone-call

- Confirm patient appointment time/date/transport
- Remind them of 6MWT/ETT requirements of no cigarettes that day

V4Pre	**Complete reminder phone-call checkbox on front of CRF**	☐

Visit 4

V4A	**Visit 3 blood results documented**	☐
V4B	**Medications log updated**	☐
V4C	**Adverse event log updated**	☐

Medication compliance

Number of tablets remaining _____

Comments _____

_____

_____

V4D	**Medication compliance checked**	☐

Study medication supply

Number of tablets supplied _____

V4E	**Bottle 4 of study medication issued, complete with instructions**	☐

Instructions for selecting Gardner Protocol:

1. Turn on treadmill at back and side
2. Login to system (Username: User; Password: User)
3. Select> NEW TEST
4. Create> NEW PATIENT and Enter: Patient ID (APOSA-PAD xxx); Gender and DOB
5. Click> ACCEPT and >SELECT
6. Go to Test Info and Select> GARDNER then >OK
7. Press> PRE-TEST (Blue Button)
8. With participant straddling the treadmill, press> START TREADMILL (Button)
9. Walk for 5-10 sec max at each speed. When Pre-test completed Press> STOP TREADMILL (Red Button) and instruct participant to wait until treadmill has stopped
10. Repeat steps 7-9 for speeds of 1, 1.5 and 2mph
11. Attach ECG electrodes and connect leads to participant during the 10 minute rest period
12. With participant straddling the treadmill, press> PRE-TEST and > START TREADMILL
13. When treadmill up to speed, participant steps on
14. When second heel hits the treadmill press> EXERCISE (Button)
15. When the participant reports onset of claudication symptoms, Press> 12 LEAD (Button) (this will print an ECG). Also note when treadmill speed reaches 2mph
16. An ECG will be printed 10 seconds prior to each change in gradient
17. When participant reports Absolute Claudication press> 12 LEAD (Button) then >RECOVERY or >TEST END. Make sure to always press> 12 LEAD before stopping test.
18. Print only CONFIGURED TEST
19. Do not save ECG data

Progress ETT

Follow instructions as per protocol on opposite page.

Claudication onset @ ___min : ___sec
Peak walking time (PWT) @ ___min : ___sec
Grade at PWT: ____

Primary reason for stopping the treadmill:

❑ Claudication

❑ Other, specify _____

Leg which was affected most during the test: ❑ Right ❑ Left

Description of claudication symptoms:

Right _____ (e.g., calf)	❑ Fatigue ❑ Cramp ❑ Pain ❑ Other, describe _____
Left _____ (e.g., calf)	❑ Fatigue ❑ Cramp ❑ Pain ❑ Other, describe _____

V4F	**Progress ETT carried out and results printed & filed in CRF**	

Whilst the participant is resting from the ETT and prior to 6 minute walk test being carried out the two study questionnaires should be completed:

V4G	**Administer Walking Impairment questionnaire**	
V4H	**Administer SF-36 Quality of Life questionnaire**	

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Six Minute Walk Test

Ten minute rest period pre-test ❑

Pre-test resting HR _____ bpm Under 120bpm? ❑

Pre-test resting BP _____ /_____ mmHg Under 180mmHg systolic & 100mmHg diastolic? ❑

Pre-test fatigue level on Borg scale _____

Number of 30m laps completed _____ = ____ x 30m = _____m total distance

Post-test fatigue level on Borg scale _____

If stopped prematurely

Time walked ____min ____ sec

Reason for early end of test: _____

_____

V4I	**Progress 6MWT carried out**	

Final Visit 4 checks

V4J	**Date for fifth visit booked and recorded on front of CRF**	

Signed		**Name**		**Date**	

Study visits overview

- Visit 1 (week 0) – screening visit 1
  - Participant consent – answer any outstanding questions and complete consent form.
  - Baseline ABI
  - Baseline ETT
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Record list of current medications

- Visit 2 (week 0) – screening visit 2
  - Second baseline ETT – if stable (<25% variance) then can continue in study
  - Six minute walk test
  - FMD measurement
  - Supply of initial study medication to participant along with instructions.
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

- Visit 3 (week 6) – progress visit
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/isoprostanes/oxidised LDL
  - Record list of current medications
  - Supply of full dose study medication (first half)

- Visit 4 (week 12) – progress visit
  - ETT
  - Six minute walk test
  - Assess medication compliance.
  - Check for AEs
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications
  - Supply of full dose study medication (second half)

- **Visit 5 (week 18) – progress visit**
  - **FMD measurement**
  - **Assess medication compliance.**
  - **Check for AEs**
  - **Bloods for FBC/UE/LFT**
  - **Record list of current medications**

- Visit 6 (week 24) – final visit
  - FMD measurement
  - ETT
  - Six minute walk test
  - ABI measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

APOSA-PAD Subject ID S.....R..... Initials ..... Date: __/__/_____ Visit 5

Pre Visit 5 phone-call

- Confirm patient appointment time/date/transport
- Remind them of FMD requirements:
  - not eating for four hours before attending
  - no tea/coffee that day
  - no cigarettes that day

V5Pre	**Complete reminder phone-call checkbox on front of CRF**	

Visit 5

V5A	**Medications log updated**	
V5B	**Adverse event log updated**	
V5C	**FMD measurements carried out**	

Medication compliance

Number of tablets remaining _____

Comments _____

_____

_____

V5D	**Medication compliance checked**	

Venepuncture

Samples required:

- 1x EDTA (purple 4ml) – FBC
- 1x clotted (yellow 5ml) – UE/LFT

V5E	**Blood samples taken for FBC/UE/LFT**	

Final Visit 5 checks

V5F	**Date for final visit booked and recorded on front of CRF**	

Signed		**Name**		**Date**	

Study visits overview

- Visit 1 (week 0) – screening visit 1
  - Participant consent – answer any outstanding questions and complete consent form.
  - Baseline ABI
  - Baseline ETT
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Record list of current medications

- Visit 2 (week 0) – screening visit 2
  - Second baseline ETT – if stable (<25% variance) then can continue in study
  - Six minute walk test
  - FMD measurement
  - Supply of initial study medication to participant along with instructions.
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

- Visit 3 (week 6) – progress visit
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/isoprostanes/oxidised LDL
  - Record list of current medications
  - Supply of full dose study medication (first half)

- Visit 4 (week 12) – progress visit
  - ETT
  - Six minute walk test
  - Assess medication compliance.
  - Check for AEs
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications
  - Supply of full dose study medication (second half)

- Visit 5 (week 18) – progress visit
  - FMD measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT
  - Record list of current medications

- Visit 6 (week 24) – final visit
  - FMD measurement
  - ETT
  - Six minute walk test
  - ABI measurement
  - Assess medication compliance.
  - Check for AEs
  - Bloods for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL
  - Walking Impairment Questionnaire
  - Quality of life (SF-36 questionnaire)
  - Record list of current medications

Pre Visit 6 phone-call

- Confirm patient appointment time/date/transport
- Remind them of FMD requirements:
  - not eating for four hours before attending
  - no tea/coffee that day
  - no cigarettes that day

V6Pre	**Complete reminder phone-call checkbox on front of CRF**	☐

Visit 6

V6A	**Visit 5 blood results documented**	☐
V6B	**Medications log updated**	☐
V6C	**Adverse event log updated**	☐

Medication compliance

Number of tablets remaining _____

Comments _____

_____

_____

V6D	**Medication compliance checked**	☐
V6E	**FMD measurements carried out**	☐

Venepuncture

Samples required:

- 1x EDTA (purple 4ml) – FBC
- 1x EDTA (pink 10ml) – isoprostanes
- 1x EDTA (pink 5ml) – oxidised LDL
- 2x clotted (yellow 5ml) – UE/LFT/uric acid, storage

V6F	**Blood samples taken for FBC/UE/LFT/uric acid/isoprostanes/oxidised LDL/storage**	☐

Instructions for selecting Gardner Protocol:

1. Turn on treadmill at back and side
2. Login to system (Username: User; Password: User)
3. Select> NEW TEST
4. Create> NEW PATIENT and Enter: Patient ID (APOSA-PAD xxx); Gender and DOB
5. Click> ACCEPT and >SELECT
6. Go to Test Info and Select> GARDNER then >OK
7. Press> PRE-TEST (Blue Button)
8. With participant straddling the treadmill, press> START TREADMILL (Button)
9. Walk for 5-10 sec max at each speed. When Pre-test completed Press> STOP TREADMILL (Red Button) and instruct participant to wait until treadmill has stopped
10. Repeat steps 7-9 for speeds of 1, 1.5 and 2mph
11. Attach ECG electrodes and connect leads to participant during the 10 minute rest period
12. With participant straddling the treadmill, press> PRE-TEST and > START TREADMILL
13. When treadmill up to speed, participant steps on
14. When second heel hits the treadmill press> EXERCISE (Button)
15. When the participant reports onset of claudication symptoms, Press> 12 LEAD (Button) (this will print an ECG). Also note when treadmill speed reaches 2mph
16. An ECG will be printed 10 seconds prior to each change in gradient
17. When participant reports Absolute Claudication press> 12 LEAD (Button) then >RECOVERY or >TEST END. Make sure to always press> 12 LEAD before stopping test.
18. Print only CONFIGURED TEST
19. Do not save ECG data

Final ABI

Remember: ABI= highest ankle pressure for each side ÷ highest Brachial BP

	Right	*Left*
Brachial BP		
Posterior tibial BP		
Dorsalis pedis BP		
ABI		

Final ETT

Follow instructions as per protocol on opposite page.

Claudication onset @ ___min : ___sec
Peak walking time (PWT) @ ___min : ___sec
Grade at PWT: ____

Primary reason for stopping the treadmill:

❑ Claudication

❑ Other, specify _____

Leg which was affected most during the test: ❑ Right ❑ Left

Description of claudication symptoms:

Right _____ (e.g., calf)	❑ Fatigue ❑ Cramp ❑ Pain ❑ Other, describe _____
Left _____ (e.g., calf)	❑ Fatigue ❑ Cramp ❑ Pain ❑ Other, describe _____

V6G ABIs recorded; ETT carried out and results printed & filed in CRF	

Whilst the participant is resting from the ETT and prior to 6 minute walk test being carried out the two study questionnaires should be completed:

V6H Administer Walking Impairment questionnaire	

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V6I	Administer SF-36 Quality of Life questionnaire	

Six Minute Walk Test

Ten minute rest period pre-test ❑

Pre-test resting HR _____ bpm Under 120bpm? ❑

Pre-test resting BP _____/_____ mmHg Under 180mmHg systolic & 100mmHg diastolic? ❑

Pre-test fatigue level on Borg scale _____

Number of 30m laps completed _____ = ____ x 30m = _____m total distance

Post-test fatigue level on Borg scale _____

If stopped prematurely

Time walked ____min ____ sec

Reason for early end of test: _____

_____

V6J	Final 6MWT carried out	

Final Visit checks

V6K	Visit 6 blood results documented	

V6L	Completion of study form (on next page) filled out	

Signed		Name		Date	

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APOSA-PAD Subject ID S.....R..... Initials ..... Date: __ /__ /_____

Completion of Study/Early Withdrawal Form

Completion

Did the subject complete the study? Yes ❑ No ❑

Date of completion/withdrawal: ____/____/_____

If subject did not complete, give reason:

_____

_____

_____

_____

Follow-up

Is any follow-up required? Yes ❑ No ❑

If so, provide details:

_____

_____

_____

Protocol

Were there any deviations from protocol? Yes ❑ No ❑

If so, provide details:

_____

_____

_____

Signed		Name		Date	

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APOSA-PAD Subject ID S.....R..... Initials ..... Date: __ /__ /_____

Medications Log

ALLERGIES:

Medication	*Indication*	*Dose*	*Frequency*	*Route*	*Start date (if known)*	*End date (if discontinued)*

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Blood results

	Visit 1	Visit 3	Visit 5	Visit 6
Haemoglobin (g/dL)				
WCC				
Neutrophils				
Platelets				
Sodium (mmol/L)				
Potassium (mmol/L)				
Urea (mmol/L)				
Creatinine (mmol/L)				
eGFR (ml/L)				
ALT (U/L)				
Bilirubins (µmol/L)				
Alk Phos (U/L)				
Albumin (g/L)				
Uric acid				
Isoprostanes				
Oxidised LDL				

Instructions for FMD measurement

Setup

- Switch on all power buttons for machine
- Connect ECG electrodes
- Press *Begin/End* to enter study screen
- Enter patient details (remember to change to brachial & FMD8L5; use first letter of first name & surname, and study number)
- Begin imaging
- Increase Edge to 3
- Press *D-colour* (increase size of the square)

Pressure

- Wrap cuff round arm (below ACF), ensure BP machine set to 200mmHg & rubber stopper in place on spare line.
- Find image of brachial artery above ACF (hold probe so marker is to the right)
- Fix snake arm (adjust twiddler to 5 to allow for some leeway)
- Move *Position* button so arrow next to artery
- Press *PW*
- Press *Angle* and move the cursor so it is inbetween the artery walls
- Then press *Gate* to move the cursor into the middle
- Then press *Store Image* when full PW can be seen across screen
- Increase *Baseline* (press it downwards) then come out of that by pressing *PW*
- Move arrow to point out where artery is (use *Position* key)
- Press *Protocol* then toggle to *Baseline*
- Press *Trigger* (top of keyboard) then *Clip store*
- Record for 1 minute
- Press *Clip store* then *Trigger*
- **WARN PATIENT FIRST!** Then inflate the cuff by pressing cuff machine foot pedal and *Protocol* at the same time
- The screen should show *Cuff inflated* (if not then toggle to this using the *Page* button next to *Protocol*)
- Fix image if required
- Leave cuff inflated for 5 minutes
- **WARN PATIENT FIRST!** Deflate cuff by pressing cuff machine foot pedal again
- Press *Protocol* the *Page* to *Post 1*
- Press *PW* then *Image store*
- Press *Trigger* and *Clip store*
- Record for 2 minutes
- Then press *Clip store* then *Trigger* again (to switch it off)
- Let the arm rest for a few minutes, switch off cuff machine.

GTN

- Find image (hold probe so marker is to the right) – remember this can be totally different to the image used for the BP part.
- Fix snake arm (adjust twiddler to 5 to allow for some leeway)
- Move *Position* button so arrow next to artery
- Press *PW*
- Press *Angle* and move the cursor so it is inbetween the artery walls
- Then press *Gate* to move the cursor into the middle
- Then press *Store Image* when full PW can be seen across screen
- Increase *Baseline* (press it downwards) then come out of that by pressing *PW*
- Move arrow to point out where artery is (use *Position* key)
- Press *Protocol* then toggle to *Baseline*
- Press *Trigger* then *Clip store*
- Record for 1 minute
- Press *Clip store* then *Trigger*
- Spray 2 puffs of GTN under tongue
- Press *Protocol* to *Post-2*
- Wait for 2 minutes then Press *PW* then *Image store*
- Press *Trigger* and *Clip store*
- Record for 2 minutes
- Then press *Clip store* then *Trigger*
- Remove cuff, this is the end of the test.

Export
Select study utility (top left). Click on study (should be 30-60MB. Insert MO disk. Click copy. Star will disappear from study once successfully copied. Eject MO disk.

FMD results grid

Visit 2

Pulse wave	Int 1	Max 1	Int 2	Max 2	Int 3	Max 3	Ave Int	Ave Max
Pre-cuff								
Post-cuff								
% change							%	%

Diameter	FMD Cuff	FMD GTN
Pre	mm	mm
Post	mm	mm
% change	%	%

Visit 5

Pulse wave	Int 1	Max 1	Int 2	Max 2	Int 3	Max 3	Ave Int	Ave Max
Pre-cuff								
Post-cuff								
% change							%	%

Diameter	FMD Cuff	FMD GTN
Pre	mm	mm
Post	mm	mm
% change	%	%

Visit 6

Pulse wave	Int 1	Max 1	Int 2	Max 2	Int 3	Max 3	Ave Int	Ave Max
Pre-cuff								
Post-cuff								
% change							%	%

Diameter	FMD Cuff	FMD GTN
Pre	mm	mm
Post	mm	mm
% change	%	%

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Adverse Events Log

Description of adverse event (provide additional information on notes pages if required)	**Date of onset**	**Date reported**	**Severity** 1. Mild 2. Moderate 3. Severe	**Relationship to IMP** 1. Unrelated 2. Possible 3. Probable 4. Definite 5. Suspected 6. Unknown	**Action taken** 1. None 2. IMP dose reduced/temp withheld 3. IMP stopped 4. Con. meds commenced 5. Other	**Date resolved** (if not resolved at end of study mark as ongoing)	**Outcome** 1. Recovered 2. Ongoing 3. SAE reported * 4. Unknown * If the AE led to the SAE, record here, complete an SAE form and fax to the sponsor

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Associated documentation

File these documents in the following order after this page:

1. Visit/contact notes
2. Visit 1 Baseline ETT printout
3. Visit 2 Second baseline ETT printout
4. Visit 2 Walking Impairment Questionnaire
5. Visit 2 SF-36 Questionnaire
6. Visit 4 Progress ETT printout
7. Visit 4 Walking Impairment Questionnaire
8. Visit 4 SF-36 Questionnaire
9. Visit 6 Final ETT printout
10. Visit 6 Walking Impairment Questionnaire
11. Visit 6 SF-36 Questionnaire
12. Blood result printouts
13. (spare)
14. (spare)
15. (spare)

K. Post-trial follow-up letters to participants

UNIVERSITY OF DUNDEE

Medical Research Institute

DIVISION OF CARDIOVASCULAR AND DIABETES MEDICINE

Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

5 July 2013

01382 632180

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

I just wanted to take this opportunity to thank you for your participation in the study over the past six months - it is greatly appreciated.

I will be in touch again in late June/early July to let you know whether you were on the active drug or the placebo. I'll then be in touch again later in the year with the final results of the study – this will take a number of months though, as I need to wait for all patients to complete their participation before I can begin to analyse the results.

Please do not hesitate to get in touch in the meantime should you have any queries or concerns.

Kind regards
Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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Head of Division and Professor of Cardiovascular Medicine
Professor Allan D Struthers BSc MD FRCP FESC FRSE FMedSci

5 July 2013

01382 383013

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

Thank you once again for taking part in this study. All patients have now completed their participation and final data entry has now taken place. As mentioned in my previous letter at this stage I wanted to let you know whether you were on the active drug (allopurinol) or the placebo. I would like to emphasise that whichever you were on your participation was equally valuable and useful – by the nature of the research, 25 patients were on allopurinol and 25 were on placebo.

In your case you were on: **«IMPPLACEBO»**

It will take a few months to fully analyse the results and write up the findings – however as soon as this is done I'll be in touch again with the final results of the study.

Please do not hesitate to get in touch in the meantime should you have any queries or concerns.

Kind regards
Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

CC «GP», «GP_Address_1», «GP_Address_2», «GP_Address_3», «GP_Postcode»

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Head of Division and Professor of Cardiovascular Medicine
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5 July 2013

01382 632180

«AddressBlock»

«GreetingLine»

Allopurinol as a possible oxygen sparing agent during exercise in peripheral arterial disease

In my last letter I promised to let you know the outcome of the analysis of the study findings. My apologies for the delay in getting this to you - unfortunately with the return to full-time clinical work this has taken longer than I hoped.

In summary, the main finding of the study was that allopurinol does not help improve walking distance in patients with intermittent claudication. Although the results were negative, they were at least clear and this does help add to our knowledge about this condition.

I would like to take this opportunity to thank you once again for taking part in the above research study - your participation was greatly appreciated.

Should you have any questions feel free to get in touch.

Kind regards
Yours sincerely

Dr Alan J Robertson
BHF Research Fellow

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